Acetamiprid

DOCUMENT M-CA, Section 9

LITERATURE DATA

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
31 October 2014	Final version, all sections completed	Final

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CA 9 LITERATURE DATA

CA 9.1 Introduction

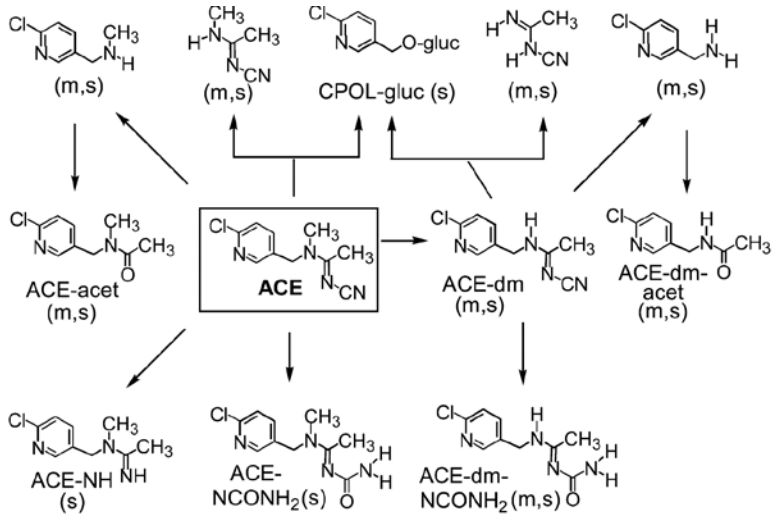
This document compiles summaries of relevant articles identified through the literature search presented in the report KCA Section 9, conducted in accordance with Article 8 (5) of Regulation (EC) No. 1107/2009. Relevance and reliability of articles found in the search process were appraised in adherence with EFSA guidelines (EFSA Journal 2011;9(2):2092 and EFSA supporting publication 2013:EN-511).

For acetamiprid, its metabolites and appropriate trade names, the review of the published literature identified 46 articles of relevance to the residues, toxicology and ecotoxicology parts of the regulatory data package. These 46 articles are summarised and presented below. The full articles are located with the literature data at KCA9.

CA 9.2 Study summaries of relevant articles

CA 9.2.1 Residues

Comparative metabolism and pharmacokinetics of seven neonicotinoid insecticides in spinach	
KCA 6.2.1	
Author(s)	Ford, K.A., Casida, J.E.
Year	2008
Journal	J. Agric. Food Chem. Vol. 56, pp. 10168–10175
Relevance check	Relevant - Limitations: Study does not follow OECD guidelines.
Reliability check	2 (Klimisch et al., 2007)
Reasons for no reliability	—
Summary	The metabolism of seven commercial neonicotinoid insecticides was compared in spinach seedlings (*Spinacia oleracea*) using HPLC-DAD and LC-MSD to analyse the large number and great variety of metabolites. The parent neonicotinoid levels in the foliage following hydroponic treatment varied from differences in uptake and persistence. The metabolic reactions included nitro reduction, cyano hydrolysis, demethylation, sulfoxidation, imidazolidine and thiazolidine hydroxylation and olefin formation, oxadiazine hydroxylation and ring opening and chloropyridinyl dechlorination. The identified phase I plant metabolites were generally the same as those in mammals, but the phase II metabolites differed in the conjugating moieties. Novel plant metabolites were various neonicotinoid-derived *O*- and *N*-glucosides and -gentiobiosides and nine amino acid conjugates of chloropyridinylcarboxylic acid. Metabolites known to be active on nicotinic acetylcholine receptors included the desnitro- and descyanoguanidines and olefin derivatives. The findings highlight both metabolites common to several neonicotinoids and those that are compound specific.

Reliability check: study details	
Parameter	**Information available**
Test crop	Spinach (*Spinacia oleracea*)
Growth stage	4 inch seedlings
Test location	University of California, Berkeley, California
Season(s)	Not applicable
Test conditions	
Application rate:	Hydroponic application of 50 mL of a 100 ppm solution of the active substance.
Number of applications:	Compound loading experiments: continuous exposure for 13 d Dissipation experiments: continuous exposure for 3 d
Pre-harvest interval:	Dissipation experiments: 10 d
Sampling	Whole leaves
Storage	Not reported
Analysis	HPLC-DAD, LC-MS
Results	For acetamiprid (ACE), the parent molecule was fairly persistent with half of the day 0 level present at day 10. Metabolism involved several initial sites of attack: N-demethylation to ACE-dm, the most prominent metabolite of those analysed; cyano hydrolysis to ACE-$NCONH_2$; cleavage of the N-CN linkage to ACE-NH; hydroxylation at the *N*-methylene substituent to yield the cyanoamidine-containing fragment and ultimately CPOL-gluc; cleavage at the $N(CH_3)$-$C(CH_3)$=N linkage to *N*-methylchloropyridinylmethylamine, which was acetylated to ACE-acet. ACE-dm underwent similar pathways to acetamiprid to yield ACE-dm-$NCONH_2$, chloropyridinylmethylamine and its acetyl derivative ACE-dm-acet and CPOL-gluc, plus the corresponding cyanoamidine-containing fragment.  For acetamiprid, the identified plant metabolites were generally the same as those in mammals, with exception of CPOL that was conjugated as the glucoside in the plant.

Overall assessment	Study provides detailed information on the metabolic pathway of acetamiprid and six other neonicotinoid insecticides in spinach and a comparison with the metabolic pathway in mice.

Effect of home processing on the distribution and reduction of pesticide residues in apples	
KCA 6.5.3	
Author(s)	Kong, Z., Shan, W., Dong, F., Liu, X., Xu, J., Li, M., Zheng, Y.
Year	2012
Journal	Food Additives and Contaminants Vol. 29, No. 8, pp. 1280–1287
Relevance check	Relevant - Limitations: Application rate much lower than cGAP. The production of apple juice and pomace is performed after peeling and coring that is not common industrial standard. Study also contains a method validation for whole fruit and juice with the deficiency of no method confirmation.
Reliability check	2 (Klimisch et al., 2007)
Reasons for no reliability	—
Summary	The effect of home processing (washing, peeling, coring and juicing) on residue levels of chlorpyrifos, ß-cypermethrin, tebuconazole, acetamiprid and carbendazim in apple segments was investigated. The pesticide residues were determined by UPLC-MS/MS and GC with a flame photometric (FPD) and electron capture detection (ECD). The results indicated that the pesticide residue levels in the apple peel and core were higher compared with in the apple flesh. After peeled and cored apple was processed into apple juice and pomace, chlorpyrifos, ß-cypermethrin and tebuconazole were concentrated in the apple pomace. However, residues of acetamiprid and carbendazim were exceptions. The apple pomace was free of acetamiprid, which was mainly present in the apple juice. After washing the mean loss of chlorpyrifos, ß-cypermethrin, tebuconazole, acetamiprid and carbendazim from apples under recommended dosage and twofold higher dosage were 17-21%, 6.7-7.1%, 13-32%, 42-67% and 47-50%, respectively. The pesticide residues were significantly reduced in the edible part of the apple except for ß-cypermethrin during peeling and coring process. The removal effect of apple juicing was found to be the most pronounced on ß-cypermethrin residue, which was reduced in the range of 81-84% and the reductions of chlorpyrifos, tebuconazole, acetamiprid and carbendazim upon apple juicing were in the range of 15-36%.
Reliability check: study details	
Parameter	**Information available**
Test crop	Apple
Test location	Beijing, China
Season(s)	Not reported
Test conditions	
Application rate:	Plot 1: 1.5 g a.s./ha (acetamiprid) Plot 2: 3 g a.s./ha (acetamiprid)

<table>
<tr><td>Number of applications:</td><td>Three applications with seven day intervals</td></tr>
<tr><td>Pre-harvest interval:</td><td>Seven days</td></tr>
<tr><td>Sampling</td><td>Apple fruits, 10 kg for each plot; approximately 1 kg of fruit (at least 10 apples) was selected from each sample and divided into two portions. One portion was washed prior to further processing whereas the other sample was left unwashed.</td></tr>
<tr><td>Storage</td><td>One day at 4°C</td></tr>
<tr><td>Analysis</td><td>UPLC-MS/MS (ESI^+) for acetamiprid</td></tr>
<tr><td>Results</td><td>The recovery of acetamiprid at various concentration levels was 83-95.4% which is in line with the range expected (Document SANCO/10684/2009).

Pesticide residues at recommended application dosage rates are as follows:

<table>
<tr><td>Processing</td><td>Products</td><td>Mean ± SD</td><td>D (%)</td></tr>
<tr><td rowspan="7">Unwashed</td><td>Whole apple</td><td>0.012 ± 0.001</td><td></td></tr>
<tr><td>Peeled and cored apple</td><td>0.003 ± 0.001</td><td>15</td></tr>
<tr><td>Apple peel</td><td>0.081 ± 0.008</td><td>66</td></tr>
<tr><td>Apple core</td><td>0.019 ± 0.002</td><td>19</td></tr>
<tr><td>Apple juice</td><td>0.008 ± 0.001</td><td>> 99</td></tr>
<tr><td>Apple pomace</td><td>< LOD</td><td>-</td></tr>
<tr><td rowspan="7">Washed</td><td>Whole apple</td><td>0.007 ± 0.001</td><td></td></tr>
<tr><td>Peeled and cored apple</td><td>0.003 ± 0.001</td><td>28</td></tr>
<tr><td>Apple peel</td><td>0.036 ± 0.002</td><td>55</td></tr>
<tr><td>Apple core</td><td>0.009 ± 0.001</td><td>17</td></tr>
<tr><td>Apple juice</td><td>0.005 ± 0.001</td><td>> 99</td></tr>
<tr><td>Apple pomace</td><td>< LOD</td><td>-</td></tr>
</table>

Pesticide residues at double recommended double application dosage rates are as follows:

<table>
<tr><td>Processing</td><td>Products</td><td>Mean ± SD</td><td>D (%)</td></tr>
<tr><td rowspan="7">Unwashed</td><td>Whole apple</td><td>0.024 ± 0.002</td><td></td></tr>
<tr><td>Peeled and cored apple</td><td>0.004 ± 0.001</td><td>21</td></tr>
<tr><td>Apple peel</td><td>0.072 ± 0.006</td><td>61</td></tr>
<tr><td>Apple core</td><td>0.017 ± 0.002</td><td>18</td></tr>
<tr><td>Apple juice</td><td>0.008 ± 0.003</td><td>> 99</td></tr>
<tr><td>Apple pomace</td><td>< LOD</td><td>-</td></tr>
<tr><td rowspan="7">Washed</td><td>Whole apple</td><td>0.008 ± 0.001</td><td></td></tr>
<tr><td>Peeled and cored apple</td><td>0.002 ± 0.001</td><td>13</td></tr>
<tr><td>Apple peel</td><td>0.071 ± 0.005</td><td>75</td></tr>
<tr><td>Apple core</td><td>0.009 ± 0.001</td><td>12</td></tr>
<tr><td>Apple juice</td><td>0.006 ± 0.001</td><td>> 99</td></tr>
<tr><td>Apple pomace</td><td>< LOD</td><td>-</td></tr>
</table>

Residues of acetamiprid were reduced in apple juice and pomace but concentrated in apple peel and core.</td></tr>
</table>

Overall assessment	Study provides detailed information of the distribution of acetamiprid and four other plant protection products in apple peel, core, juice and pomace with the limitation of not presenting data according to an industrial process of juice production.

CA 9.2.2 Toxicology

In vitro genotoxicity evaluation of acetamiprid in CaCo-2 cells using the micronucleus, comet and cH2AX foci assays	
KCA 5.4.1	
Author(s)	Çavaş, T., Çinkılıç, C., Vatan, Ö., Yılmaz, D., Coşkun, M.
Year	2012
Journal	Pesticide Biochemistry and Physiology Vol. 104, pp. 212–217
Relevance check	Relevant
Reliability check	3
Reasons for no reliability	The study was not to GLP or guideline and the choice of cell line is highly unusual and not validated in these assays. The choice of positive control was poor, as it was not a direct genotoxin. Replicates utilising a metabolic activation system (e.g. S9) were also not present.
Summary	Acetamiprid is a member of the neonicotinoid group of insecticides commonly used against wide range of insect pests. In the present study, *in vitro* cytotoxicity and genotoxicity of technical grade acetamiprid was evaluated on the human intestinal CaCo-2 cells. Cytotoxicity was evaluated using the clonogenic survival and the results indicated that acetamiprid was cytotoxic on CaCo-2 cells. The cells were than treated with acetamiprid concentrations exhibit greater than 75% clonogenic survival for 24 h, to assess genotoxicity using the micronucleus, comet and cH2AX foci formation assays. Our results indicate that, under the experimental conditions used, acetamiprid has cytotoxic and genotoxic potential on human intestinal cells.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	*In vitro* micronucleus assay Non-guideline. Non GLP. *In vitro* comet assay. Non-guideline. Non GLP.
Test substance Identification of test substance, source, purity, stability	Technical grade acetamiprid (95% pure)
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and	The human colon carcinoma cell line CaCo-2, provided by Dr. E. Ulukaya (Uludag University) was used for the experiments, at passage 30. The CaCo-2 cells were grown in RPMI-1640 medium supplemented with 15% fetal calf serum (FCS), penicillin (100 IU ml) and streptomycin (100 µg ml), 10 mM L-glutamine, 10 mM non-essential amino acids and sodium pyruvate. Cells were maintained at 37ºC in a humidified atmosphere containing 5% CO_2. Cells were

maintenance, study protocol	grown in 75 cm^2 flasks and subcultured once a week. **Cytotoxicity** Cytotoxicity was determined by a clonogenic assay which measures the reduction in plating efficiency in treatment groups relative to the controls. Eighty thousand cells were seeded into a 6 well tissue culture plate and allowed to grow for 48 h. The cultures were then treated for 24 h with serial concentrations (25, 50, 100, 150, 200, 250, 300 and 350 µM) of acetamiprid. Following exposure period, the treatment medium was collected, the cells were rinsed with PBS; and then removed with 0.25% trypsin/1 mM EDTA solution. Cells were centrifuged at 1000 rpm, 4ºC for 5 min. The resulting pellet was re-suspended in 5 ml of medium, counted with Cedex XS (Roche) cell counter and re-seeded at colony forming density (1000 cells per well). Colonies were allowed to grow for 10 days, fixed with 100% methanol, stained with crystal violet and counted. Four dishes were used for each treatment and experiments were repeated three times. **Cytokinesis-block micronucleus test** Cytokinesis-block micronucleus test was used to detect chromosomal damages occurred due aneugenic or clastogenic effects. CaCo-2 cells were seeded in sterile cell culture dishes (60 mm) at a density of 5×10^4 cells/dish and allowed 48 h to establish normal growth. Cells were then treated with different concentrations of acetamiprid and H_2O_2 for 24 h. After treatment, cells were further cultured with cytochalasin-B for 24 h. Then, cells were trypsinized centrifuged and resuspended in 0.075 M KCl and incubated for 2 min. Cells were then fixed 3 times in methanol:glacial acetic acid (3:1). Following fixation, the cell solution was dropped onto pre-cleaned slides and the nucleus was stained by 5% giemsa for 10 min. Slides were analysed and under light microscope and the number of binucleated (BNC) cells with micronuclei (MNBNC) was recorded based on observation of 2000 cells per treatment group. Cytotoxicity was further estimated by using the nuclear division index (NDI). The numbers of cells with one to four nuclei were determined in 1.000 cells. NDI was calculated using the following formula: NDI = (1 × M1+2 × M2+3 × M3+4 × M4)/1.000; where M1 through M4 represent the number of cells with one to four nuclei. **Immunofluorescence for γH2AX foci formation** γH2AX foci were used to detect the presence of acetamiprid induced double strand DNA breaks. Cells were grown on 8 well chamber slides. After treatment with acetamiprid for 24 h, the cells were fixed in 4% paraformaldehyde for 10 min, permeabilized with 0.2% Triton X-100 for 5 min and blocked with 1% BSA for 1 h. Cells were then incubated with anti-γH2AX antibody at 4ºC overnight and then incubated with a AlexaFluor 488-conjugated second antibody for 1 h. Nuclei were counterstained with DAPI. The slides were mounted and

	viewed with a Nikon Fluorescence Microscope. γH2AX foci were counted in 100 cells per treatment concentration and 4 independent experiments were conducted. **Alkaline comet assay** For the comet assay, CaCo-2 cells treated in 60 mm dishes were harvested and embedded in 0.8% low melting agarose on slides precoated with normal melting point agarose. Slides were then placed in prechilled lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris base, pH 10) with 1% Triton X for 1 h at 4ºC. Cells were then denatured in alkaline buffer (0.3 M NaCl, 1 mM EDTA) for 30 min in the dark at room temperature. Electrophoresis was performed at 25 V and 300 mA for 20 min. The slides were immersed in neutralization buffer (0.5 M Tris–HCl, pH 7.5) for 10 min followed by dehydration in 70% ethanol. The slides were air dried and stained with ethidium bromide (EtBr). Comets were analysed by visual scoring and genetic damage index (GDI) values were calculated.
Controls Positive control, negative	Positive control: H_2O_2 100 µM Negative control: Distilled water (max 0.5% v/v)
Dosing system Exposure (dose, duration, frequency)	Addition to the culture medium
Statistical analyses Sample size/replicates statistical analysis of data (significance level, variability)	Both parametric and nonparametric tests were used in order to detect differences at the 0.05 level of significance after assessing the normality of distribution of the data. Cytotoxicity data was analysed on the percentage of cells that survived compared to the control. Differences between mean values were compared using least significant difference test for the micronuclei data. T-test was used for evaluation of γH2AX data. Comet assay data distributions are generally non-Gaussian, even after logarithmic transformation, which precludes the use of parametric tests. Thus we applied non-parametric Mann–Whitney U-test which is used for evaluation of visual comet data. Regression analyses were also carried out to determine the concentration–response relationships.
Results Determined effect concentration, dose response observed	**Cytotoxicity** Acetamiprid induced cytotoxicity in a concentration-dependent manner in CaCo-2 cells. Acetamiprid induced a clear and statistically significant decrease in cell survival over a range of 75–350 µM. Concentrations of 25 and 50 µM acetamiprid induced 99 and 96% relative survival respectively ($P < 0.05$). On the other hand concentrations of 75, 100, 150, 200, 250, 300 and 350 µM acetamiprid induced 90, 86, 79, 70, 62, 50 and 37% relative survival, respectively. Based on these findings, two non-cytotoxic (25 and 50 µM) and three cytotoxic (75 and 150 and 300 µM) acetamiprid concentrations were selected for further study. **Cytokines-blocked micronucleus test**

	The frequency of MNBNC was determined in CaCo-2 cells treated with various doses of acetamiprid. The background frequency of MNBNC was 2.4‰. The MNBNC frequency increased to 10.5‰ in the H_2O_2 (100 µM) positive control group. The higher non-cytotoxic concentration of acetamiprid (50 µM) caused two fold increase in MNBNC frequency ($P<0.05$). Furthermore, all three cytotoxic concentrations of acetamiprid also significantly increased the frequency of MNBNC to 4.6‰ (75 µg/ml), 5.4‰ (150 µg/ml) and 6.2‰ (300 µg/ml). This increase in micronucleus formation was concentration-dependent ($r^2 = 0.870$, $P<0.05$). However, acetamiprid did not induce MNBNC frequencies to the same extent as positive control H_2O_2. Treatment with acetamiprid decreased nuclear division index (NDI) values at all tested concentrations. However significant results ($P<0.01$) were obtained only at the two highest cytotoxic concentrations (150 and 300 µM). Positive control H_2O_2 ($P <0.01$) also significantly decreased the NDI in CaCo-2 cells in comparison with the control group. This decrease was also concentration dependent ($r^2 = 0.900$, $P <0.05$). Comparison of NDI values induced by acetamiprid and H_2O_2 revealed that acetamiprid did not reduce the NDI to the same extend as the positive control. **Comet assay** Treatment with 25, 50, 75, 150 and 300 µM doses of acetamiprid significantly increased the control GDI value of 0.3 to 0.43, 0.51, 0.59, 0.67 and 0.88, respectively ($P <0.05$). Treatment with positive control H_2O_2 also significantly increased GDI value in CaCo-2 cells ($P <0.001$). This increase was concentration-dependent ($r^2 = 0.970$). No significant increase in DNA damage the solvent control group was observed ($P>0.05$). The GDI value induced by the highest concentration of acetamiprid (0.88) was similar to that of positive control (1.29). **γH2AX foci formation** DNA double strand breaks were measured as the formation of γH2AX foci. The number of γH2AX foci per cell significantly and concentration dependently ($r^2 = 0.892$) increased with acetamiprid concentrations from 75, 150 ($P < 0.05$) to 300 µM ($P < 0.01$). No significant increase at non-cytotoxic concentrations was determined. Treatment with positive control H_2O_2 also significantly induced double strand DNA breaks as revealed by a sharp increase in the number of γH2AX foci ($P < 0.001$). However, acetamiprid did not induce γH2AX foci formations to the same extent as positive control H_2O_2. **Discussion** In the present study, we investigated the genotoxic effects of

	acetamiprid on CaCo-2 cells using three different test systems. To our knowledge this is the first study examining the in vitro cytotoxicity and genotoxicity of technical grade acetamiprid. In our study we examined the effects of cytotoxic (75, 150 and 300 µM) and non-cytotoxic (25 and 50 µM) concentrations of acetamiprid. Data on the actual residue level of acetamiprid in human is very scarce. In a study performed by Todani et al., blood acetamiprid level of a man was reported as 95 µM 20 h after the onset of acute poisoning. This concentration induced approximately 90% 24 h survival on CaCo-2 cells in clonogenic assay. In our study acetamiprid treatment significantly induced the formations of MN in cytokines blocked CaCo-2 cells. A micronucleus could be originated from aneugenic or clastogenic events. In the present study, evaluation of DNA damage by the alkaline comet assay in acetamiprid treated CaCo-2 cells revealed significantly increased single strand DNA breaks at both cytotoxic and non-cytotoxic concentrations. These findings indicate the clastogenic potential of this insecticide. On the other hand, significant decreases in the NDI values were observed only at the cytotoxic concentrations. Our cytotoxicity results are in agreement with those of Kocaman and Topaktas reported reduced NDI values in acetamiprid treated human lymphocytes. In our study, DNA damaging effects of acetamiprid was further evaluated using the γH2AX foci formation assay. The γH2AX assay is a relatively newly established test system that measures double strand DNA breaks. Results of both in vivo and in vitro studies revealed that it can be as sensitive as the comet assay. Furthermore, comparison of the γH2AX assay with the micronucleus test indicated that the micronucleus formation correlates well with γH2AX phosphorylation. In our study we observed significant increases in the number of γH2AX foci in per CaCo-2 cell exposed acetamiprid, indicating the double-strand DNA-damaging potential of this insecticide at higher concentrations. In our study, positive control H_2O_2 caused approximately 4–7 fold increase in formation of MNBNC as well as single and double DNA strand breaks in comparison with their background levels. Similar increase ratios were reported in previous studies with CaCo-2 cells. **Conclusion** The results clearly demonstrated the in vitro cytotoxicity and genotoxicity of technical grade acetamiprid on CaCo-2 cells. Our results further indicated that the cH2AX foci assay can be used as a complementary assay in assessment of in vitro pesticide genotoxicity. We also suggest that in vivo and/or in situ genotoxicity studies should also be performed to acquire a comprehensive knowledge of the acetamiprid genotoxicity.

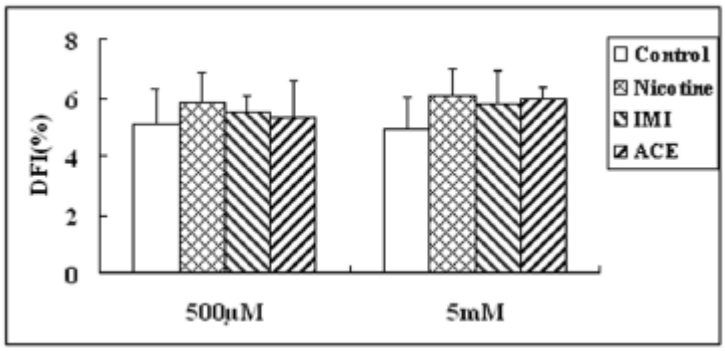
Overall assessment	The study was not to GLP or Guideline. Although well reported the study was not conducted in a validated cell line. The choice of positive control was also poor as it is not a direct genotoxic agent, but rather a mediator of oxidative stress. This study cannot supersede GLP and guideline compliant studies conducted for the registration of acetamiprid.

Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development *In vitro*	
KCA 5.6	
Author(s)	Gu, Y., Li, Y, Huang, X., Zheng, J., Yang, J., Diao, H., Yuan, Y., Xu, Y., Liu, M., Shi, H., Xu, W.
Year	2013
Journal	PLoS one July 2013, Volume 8, Issue 7, e70112
Relevance check	Relevant
Reliability check	3
Reasons for no reliability	An *in vitro* study not relevant to actual gamete exposures, relevance of concentrations tested not justified and way in excess of *in vivo* exposures, (with the lower concentration being defined as that for nicotine which caused massive fragmentation and death the next day) and exposure to neat material rather than metabolites as would occur *in vivo*.
Summary	Acetamiprid (ACE) and imidacloprid (IMI) are two major members in the family of neonicotinoid pesticides, which are synthesized with a higher selectivity to insects. The present study determined and compared *in vitro* effects of ACE, IMI and nicotine on mammalian reproduction by using an integrated testing strategy for reproductive toxicology, which covered sperm quality, sperm penetration into oocytes and preimplantation embryonic development. Direct chemical exposure (500 μM or 5 mM) on spermatozoa during capacitation was performed and *in vitro* fertilization (IVF) process, zygotes and 2-cell embryos were respectively incubated with chemical-supplemented medium until blastocyst formation to evaluate the reproductive toxicity of these chemicals and monitor the stages mainly affected. Generally, treatment of 500 μM or 5 mM chemicals for 30 minutes did not change sperm motility and DNA integrity significantly but the fertilization ability in IVF process, indicating that IVF process could detect and distinguish subtle effect of spermatozoa exposed to different chemicals. Culture experiments in the presence of chemicals in medium showed that the fertilization process and zygotes are adversely affected by direct exposure ($P<0.05$), in an order of nicotine>IMI>ACE, whereas developmental progression of 2-cell stage embryos was similar to controls ($P<0.05$). These findings unveiled the hazardous effects of neonicotinoid exposure on mammalian sperm fertilization ability as well as embryonic development, raising the concerns that neonicotinoid pesticides may pose reproductive risks on human reproductive health,

	especially in professional populations.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Technique used mimicked those employed in the human *in vitro* fertilisation (IVF) procedure. Not GLP.
Test substance Identification of test substance, source, purity, stability	Acetamiprid (>96% pure), Imidacloprid (>96% pure)
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	**Animals** 6–8 weeks old female B6D2F1 (C57BL/6xDBAx2) strain mice were used as oocyte donors and 10–15 weeks old male B6D2F1 mice were used as semen donors. All mice were housed under controlled light conditions (12 h light: 12 h dark) in the Laboratory Animal Services Facility and were fed a standard mouse diet and water ad libitum. **Experimental design** To investigate the effect of the three test materials on fertilization and embryonic development, concentrated ACE, IMI and nicotine were prepared as medium supplements. DMSO was used at final concentration ≤0.1% and this vehicle was used as control to investigate the potential effect of the solvent. In the preliminary experiment, nicotine exposure at 500 µM for 30 minutes did not impair either motility and fertilization capability of mouse spermatozoa. Concentrations of 5 mM were then adopted for the sperm exposure experiment. In sperm exposure experiment, mouse spermatozoa were placed in ACE, IMI or nicotine-containing (500 µM or 5 mM) HTF medium supplemented with bovine serum albumin for 30 min first, then washed by and incubated in fresh HTF-BSA medium for another 60 min until capacitation finished, followed by normal IVF procedure. Control spermatozoa were processed with the same procedure except the exposure of chemicals. To study their effects on the development of early embryos that skipped the stage of fertilization or the first cleavage, zygotes with two pronuclei as well as 2-cell stage embryos by natural insemination were cultured in ACE, IMI or nicotine-added KSOM medium (500 µM) to observe how chemicals worked at subsequent developmental stage. Furthermore, the consecutive exposure process from fertilization to blastocyst formation was monitored with exposure concentration of 500 µM both in HTF medium for fertilization and KSOM medium for embryo culture. Concentrations of pesticides were limited to 500 µM because the preliminary experiments indicated that oocytes and embryos with higher than 500 µM of nicotine would induce massive fragmentation or death the

	next day. **Collection of Spermatozoa** Caudal epididymides were isolated, gently squeezed out and placed in a 2 ml eppendorf tube with HTF-BSA. 'Swim-up' spermatozoa were obtained after incubation at 37ºC for 10 min. **Collection of Oocytes and Embryos** Mature female mice were superovulated with 10 IU of pregnant mare serum gonadotropin (PMSG) and 5 IU of human chorionic gonadotropin (HCG) at 48 h intervals. 14–16 h after HCG administration, cumulus oocyte complexes (COCs) were collected from the removed oviducts and then maintained in human tubal fluid medium supplemented with 10% human serum albumin at 37ºC in an atmosphere of 5% CO_2 in air until use. With regard to the recovery of the naturally fertilized zygotes and embryos, several female mice were mated with males and examined 12–18 h after HCG injection for the presence of copulation plugs. Fertilized oocytes and 2-cell embryos were recovered by flushing the oviducts 24 h and 40 h later after the HCG injection, respectively. The cumulus of oocytes were dispersed with 0.1% hyaluronidase and washed in several changes of HCZB medium. Fertilized oocytes (identified by the presence of a second polar body and two pronuclei) and 2-cell embryos were then placed in potassium chloride supplemented simplex optimized medium, which was designed for culture of implantation stage embryos and previously equilibrated in a humidified atmosphere of 5% CO_2 in air at 37ºC. **Sperm Motility Assay** The control droplet consisted of an equivalent volume of DMSO in treated groups. After incubation, a 15 µl aliquot of the treated and control samples was transferred into each of two compartments on a glass cannula slide for computer-assisted sperm analysis (CASA) using the integrated visual optical system (IVOS) motility analyser. Thirty frames were acquired at a frame rate of 60 Hz. The operational settings of the IVOS were as follows: minimum contrast (40) and size (four pixels), gate thresholds 0.38/1.65 for intensity and 0.42/2.34 for size, static elongation 0/75, progressive minimum path velocities of sperm (VAP) 50 µm/sec, straightness threshold 50% and magnification 0.82. **Sperm Chromatin Dispersion (SCD) Assay** Generally, SCD assay was developed as the HalospermH kit instructed. An aliquot of each semen sample was diluted to 5–10 million/ml in PBS. The unfixed suspensions were mixed with 1% low-melting-point aqueous agarose (to obtain a 0.7% final agarose concentration) at 37ºC. Aliquots of 20 µl mixture were pipetted onto a

	glass slide precoated with 0.65% standard agarose, covered with a coverslip (22 x 22 mm) and left to solidify at 4ºC for 5 min. Then coverslips were carefully removed and slides immediately incubated with freshly prepared acid denaturation solution for 7 min (RT) in the dark to generate restricted single-stranded DNA (ssDNA) motifs from DNA breaks. The denaturation was then stopped, followed by incubation with lysing solution for 23 min (RT). Slides were thoroughly washed in deionized water for 5 min, dehydrated in sequential 70%, 90% and 100% ethanol baths (2 min each) and air dried. Afterwards, cells were stained with modified Wright-Giemsa stain for bright-field microscopy and a minimum of 400 spermatozoa per sample were evaluated under the x 40 objective of the light microscope. After staining, four SCD patterns were established: sperm heads with (i) large size halos, whose halo width was similar or larger than the minor diameter of the core, (ii) medium size halos, whose halo size was between those with large and with small halo, (iii) small size halos, whose halo width was similar or smaller than one third of the minor diameter of the core and (iv) without a halo or degraded sperm cells, the latter ones were weakly or irregularly stained. The spermatozoa without DNA damage showed nucleoids with large- or medium-sized halos of spreading DNA loops whereas those with fragmented DNA appeared with a small or no halo. Finally, the percentage of sperm (iii) and (iv) was considered as DNA fragmentation index (DFI) for each semen sample. In this study, spermatozoa pre-incubated in ACE, IMI or nicotine-added HTF medium (5 mM or 500 µM) for 30 min were analysed for DNA integrity. ***In vitro* Fertilization and Preimplantation Embryonic Development Procedure** IVF procedure was performed as previously described (Wakayama *et al*, 2009). HTF medium was equilibrated in a 37ºC, 5% CO_2 incubator one day before experiment. Next day, caudal epididymides were collected from adult male mice. A dense sperm mass was squeezed out and then incubated in HTF-BSA medium for 60–90 min at 37ºC to develop their fertilization potential (capacitation). A small volume of capacitated sperm suspension was added to a drop of 200 µl HTF-BSA medium containing freshly ovulated oocytes to achieve a final sperm concentration of 10^6/ml. Four to six hours later, fertilized oocytes at pronuclear stage were washed and cultured in KSOM for *in vitro* development to morula/blastocyst stages in 5% CO_2 in air. Oocytes were observed for male and female pronucleus formation (fertilization) at 6 h after the initiation of culture and the number of 2-cell embryos, 4-cell embryos, morulae and blastocysts after 24, 48, 72 and 96 h in culture were checked and recorded.
Controls Positive control, negative	DMSO (negative control) Nicotine (positive control)

Dosing system Exposure (dose, duration, frequency)	Addition to culture media. 5mM, 500µM, Sperm and oocytes were exposed for 30 minutes. Concepti were exposed for 96 h.
Statistical analyses Sample size/replicates statistical analysis of data (significance level, variability)	**Statistical Analysis** SPSS for Windows (Version 15.0) was used for statistical analysis. *In vitro* developmental outcomes and SCD results were evaluated using Chi^2 tests and one-way analysis of variance (ANOVA) for significance, respectively. Results were considered statistically significant at $P<0.05$.
Results Determined effect concentration, dose response observed	**Influences of Chemical Exposure on Sperm Function** With CASA, objective and quantitative descriptions of changes in sperm kinematic parameters were obtained in response to exogenous toxicant. Treated with 500 µM or 5 mM of ACE or IMI for 30 minutes, motility of spermatozoa showed no obvious difference from that of control. Any toxicant-induced reproductive hazards associated with sperm DNA lesion were then investigated using SCD assay. All treated groups displayed a minor increase in average percentage of DNA fragmented spermatozoa compared with those of control groups without reaching a significant difference ($P<0.05$), as shown in Figure 1. **Figure 1: The SCD results of toxicants exposure upon sperm DNA integrity**  With respect to the difference between 500 µM and 5 mM of each test material, the response toward exogenous compounds at current differential concentration was not obvious, as similar effects on sperm motility and DFI were observed. When IVF was performed with the spermatozoa pretreated with test materials at the concentration of 500 µM or 5 mM, all fertilized oocytes survived without evident changes in cell morphology. Fertilized oocytes were judged normal by extrusion of second polar body and the presence of two pronuclei, which represents success of fertilization. The preliminary data indicated that treatment with 500 µM of chemicals for 30 min did not induce any significant adverse effect on fertilization potential of the spermatozoa and

	subsequent embryo development. However, during the culture process, embryos originated from spermatozoa pretreated with 5 mM of chemicals were more inclined to encounter failure of the first cleavage, wherein some of them exhibited various degrees of cellular fragmentation and asymmetry. Finally, when these embryos were cultured *in vitro* up to 96 h, with fragmentation, loss of cytoplasm or decrease in cytoplasmic clarity, part of them would arrest or degenerate during the developmental progression. In the presence of 5 mM toxicant in HTF medium, all treated spermatozoa retained their potential to fertilize oocytes. However, in nicotine and IMI-exposed groups, rates of pronucleus formation (fertilization), the first cleavage and morula/blastocyst formation were remarkably decreased, compared to those of non-treated control (P<0.05). In the ACE-exposed group, the first cleavage of zygote and blastocyst formation process were also impaired (91.2% and 58.5%, respectively), compared to those of the control group (98.5% and 74.6%, respectively) whereas the fertilization rate was slightly lower than that of control without reaching a statistical significance. Thus, ACE appeared to pose much weaker adverse effects on mouse spermatozoa, at least in terms of fertilization process *in vitro*. During embryo culture, embryo fragmentation, a process where portions of the embryo's cells have broken off, was noteworthy to the author. It is preferable to have little or no fragmentation when evaluating a normal embryo, while nicotine and IMI exposure remarkably elevated the fragmented embryo percentage compared to the control. Comparisons were also made among treated groups, it was observed that ACE exerted a significantly moderate effect, whereas nicotine exposure showed the most severe reproductive hazard. **Influences of Chemical Exposure on Fertilization and Subsequent Embryonic Development *in vitro*** In consecutive exposure experiments with chemical exposure in HTF and KSOM medium, the concentration of supplementation was constricted to 500 μM in both of media, which allowed the fertilized oocytes to proceed to blastocysts without inducing excessive fragmented or dead embryos. It was shown that a mixture of oocytes and spermatozoa in chemical-added HTF maintained normal fertilization capacity compared with controls (ratio of oocytes with pronuclei formation, P<0.05), while the percentages of 2-cell embryo and morula/blastocyst formation decreased significantly (P<0.05). Among the test materials, ACE appeared weakest in the effect on the first cleavage (ratio of 2-cell embryo formation), with no significance found. **Influences of Chemical Exposure on Preimplantation Embryonic Development *in vitro*** Considering that exposure of 1 mM chemicals would cause considerable embryo fragmentation or even death in the preliminary

	experiments, 500 µM was used in culture medium. Exposure of the test materials caused a moderate decline in the percentage of 2-cell embryo formation and a drastic impact on morula/blastocyst formation derived from normal zygotes, compared with controls (P<0.05). When comparison was made among the test materials, exposure of IMI or ACE retarded embryonic development to a more moderate degree than nicotine but no significant difference existed between the IMI and ACE groups. Taken together, the adverse effects exerted on the development of zygotes are in the order of nicotine>IMI>ACE. Incubated with 500 µM of each chemical, the development of naturally fertilized 2-cell embryos was also monitored. Under these conditions, treatment with the test materials did not show significant adverse effects on 2-cell embryos (P<0.05), with less extent of effects than those observed in zygotes. These results collectively revealed that the doses up to 500 µM of each chemical used in the study did not exert toxicity at the onset of 2-cell embryo, but fertilization or zygote as well as the subsequent developmental procedure with preceding chemical exposure. **Discussion** Despite of lower affinity to mammalian nAChRs, neonicotinoids have been illustrated to impair mammalian reproduction by recent animal studies. In the present study, a set of *in vitro* models of reproductive toxicology were used and examined the direct effects of IMI and ACE, on spermatozoa, fertilization procedure and preimplantation embryo development. Sperm quality, such as motility and DNA integrity, are important in male fertility and in the particular contribution to early embryonic development, which is also a sensitive and quick testing strategy for reproductive toxicology. *In vitro* exposure of nicotine to human semen was reported to be able to cause human sperm DNA damage and motility decrease (1 mM for 20 min). However, motility and DNA integrity were not significantly affected by a high exposure dosage (5 mM for 30 min) of chemicals, even with nicotine, which may result from the difference in the experimental objects, i.e. mouse spermatozoa versus human semen. When IVF process was introduced, subtle differences among the spermatozoa caused by pretreatment with the different test materials could still be detected through the procedures of fertilization and subsequent embryonic development. The IVF procedure includes sperm-egg binding, zygote formation and the first cleavage to form 2-cell embryo. After being transferred into KSOM medium, 2-cell embryo could conduct multiple cleavages to successively form 4-cell embryo, morula then blastocyst *in vitro*. In order to determine the specific embryo developmental stages that the test materials could affect, a mixture of spermatozoa and eggs for

	fertilization, naturally fertilized zygotes and 2-cell embryos were separately prepared and consecutive chemical exposure with a concentration of 500 µM was conducted until blastocyst formation. Exposure to these materials during fertilization could adversely affect 2-cell formation and subsequent embryo development, normal zygotes with chemical exposure could impair subsequent 4-cell embryo formation and the following procedure, whereas there was no significant adverse effect on subsequent development when normal 2-cell embryos were treated with these chemicals. Compared with the effects on fertilization procedure or zygotes, the results suggested that 2-cell embryos were most resistant to exposure of 500 mM nicotine, IMI or ACE, which is consistent with the previous study of 2-cell embryos toxicity with nicotine. Although it may cause human reproduction disorder, nicotine could show *in vitro* detriments only with a concentration much higher than the exposure level in an 'average' smoker, suggesting that nicotine might adversely affect spermatozoa or embryos in an indirect way. These results supported previous reports and imply that IMI and ACE may work with a similar mechanism to nicotine. Studies indicate that acute or chronic exposure of nicotine will cause oxidative stress in animal and human body, which could do harm to reproductive organs. Several studies reported that oxidative stress caused by testicular tissue and lymphocyte in semen will impair sperm parameters, suggesting that owing to lack of lymphocytes around, 'swim-up' mouse spermatozoa in this study are more resistant to the exposure of nicotine than human spermatozoa in semen. Human exposure to neonicotinoids is very limited (12.8–350 ng/ml in the urine of farm workers, with or without protection) and this study indicated that, at exposure levels, IMI and ACE do not show adverse effects on mouse sperm functions and early embryo development *in vitro*. However, recent animal studies showed that IMI and ACE could cause oxidative stress in the body and even chronic exposure of IMI with a low concentration could result in oxidative stress in tissues, which suggests that low level of neonicotinoid exposure over a long period may also exert impact on human reproduction especially for professional populations. Taken together in a reproductive toxicity study, several *in vitro* tests were integrated and reported in this and previous studies conducted by the authors, which covered sperm quality, sperm penetration into oocytes, process of oocyte *in vitro* maturation and preimplantation embryonic development. The results indicated that, at high levels, direct exposure of nicotine, IMI or ACE had harmful effects on sperm function and embryonic development and stages mainly at fertilization, zygote formation and first cleavage of zygote, with the extent in an order of nicotine>IMI>ACE. These results elucidated the reproductive toxicities of two neonicotinoids on mammals from a

	new prospective, which evaluated the direct effects of pesticides on gametes, fertilization and embryonic development.
Overall assessment	Although this is a well described study it is an *in vitro* study not relevant to actual gamete exposures, relevance of concentrations tested not justified and way in excess of *in vivo* exposures, (with the lower concentration being defined as that for nicotine which caused massive fragmentation and death the next day) and exposure to neat material rather than metabolites as would occur *in vivo*. Superseded by existing *in vivo* data.

Two cases of acute poisoning with acetamiprid in humans	
KCA 5.9.3; KCA 5.9.5; KCA 5.9.6	
Author(s)	Imamura, T., Yanagawa, Y., Nishikawa, K., Matsumoto, N., Sakamoto, T.
Year	2010
Journal	Clinical Toxicology (2010) Vol. 48(8), pp. 851–853
Relevance check	Relevant
Reliability check	1
Reasons for no reliability	Case reports
Summary	Two cases of acute poisoning (attempted suicides) with an insecticide formulation containing acetamiprid are described. Both cases experienced severe nausea and vomiting, muscle weakness, hypothermia, convulsions and clinical manifestations including tachycardia, hypotension, electrocardiogram changes, hypoxia and thirst in the case with the higher serum concentration of acetamiprid. The symptoms were partially similar to acute organophosphate intoxication. Supportive treatments for a variety of symptoms were sufficient for recovery and both individuals were discharged without any complications two days after ingestion.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	None: Cases study. First hand reporting of patients poisoned with formulation containing acetamiprid. Other materials in the formulation are also known, allowing any known co-symptoms of poisoning with the co-formulants to be considered.
Test substance Identification of test substance, source, purity, stability	Formulation 1: Insecticide formulation containing 18.0% acetamiprid, 31.0% N-methyl-2-pyrrolidone and 47.95% dimethylsulfoxide including 3.05% surface-active agent Formulation 2: 2.0% acetamiprid and 97% diethylene glycol (DEG) with 1% surface-active agent.
Test system characterization and study design	**Case 1** A 58-year-old male patient with diabetes and diabetic gangrene escaped from the hospital where he was being treated. The patient

Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	subcutaneously injected approximately 8 mL of formulation 1 and thereafter ingested approximately 10 mL of the same agent in an attempted suicide. **Case 2** A 74 year-old female patient had ingested approximately 100 mL of formulation 2 in an attempt to commit suicide.
Controls Positive control, negative	None
Dosing system Exposure (dose, duration, frequency)	Case 1: Single self-administered exposure by two routes: 8 mL of formulation 1 by injection followed immediately by 10 mL by mouth. Case 2: Single self-administered oral dose of 100 mL of formulation 2.
Statistical analyses Sample size/replicates statistical analysis of data (significance level, variability)	None
Results Determined effect concentration, dose response observed	**Case 1** Upon injection and swallowing formulation 1, the patient immediately experienced nausea and muscle weakness. Two hours later, the patient was transported to the emergency room of the authors' hospital by an ambulance. The patient did not have any observable mental disorder. Upon his arrival at the emergency room, the patient presented with a Glasgow Coma Scale of 15 and both of the pupil diameters were within the normal range and the reactions to the lights were prompt. The patient's vital signs were unremarkable except for his body temperature (33.7°C). The patient had suffered a single, self-limiting seizure but no longer had nausea or muscle weakness at the time of admission. Evaluation of the patient's arterial blood gases revealed metabolic acidosis and the patient's electrocardiogram was normal. The patient underwent treatment with gastric lavage, activated charcoal, cathartics and antiemetics. No neurological or physical disturbances were observed after admission and the patient was transferred back to his previous hospital two days later without any serious symptoms. **Case 2** Within 90 minutes of ingestion, the patient was transported to the emergency room of the authors' hospital by an ambulance because of nausea, muscle weakness, a single self-limiting seizure, tachycardia, hypotension, dyspnea and thirst. The patient had been treated for hypertension and ventricular extrasystole in another hospital, with medication of cilnidipine and candesartan for approximately 20 years but had no history of any mental disorders. Upon arrival, the patient's Glasgow Coma Scale was 15, with normal reactive pupils. The

	patient's blood pressure was 82/40 mmHg, her pulse rate was 104 per min (irregular rhythm), her respiratory rate was 18 per min, her oxygen saturation was 84% on room air, temperature 34.4°C and urine flow more than 1 mL/hour/kg. The initial clinical data revealed metabolic acidosis with a high anion gap, hypoxia, hypokalemia and a high arterial lactate level. The patient had not taken any medications that might cause these changes, which we attributed to the DEG formulant. The initial electrocardiogram showed multiple ventricular extrasystoles and a 3-mm ST segment depression at leads II and V4-6. An echocardiograph showed normal wall motion with an ejection fraction of greater than 60%. The patient underwent treatment with gastric lavage, activated charcoal, cathartics and antiemetics. Supportive treatments for a variety of symptoms, including an H2 blocker, vasoconstrictors, oxygen, potassium and antibiotics, were administered. The patient's nausea and hypoxia lasted 7 and 20 h, respectively, after ingestion. Hypotension and tachycarida both improved by 11 h post-ingestion. The patient's thirst disappeared 22 h after ingestion and she full recovered thereafter and was discharged on the day after ingestion. **Discussion** The muscle weakness seems to be similar to acute organophosphate (OP) poisoning. It is uncertain whether the clinical features of hypothermia and convulsions were due to the active acetamiprid ingredient. The convulsions were observed on admission in both cases but rapidly resolved without any treatment. There are currently no reports that the co-formulants in either case result in hypothermia and convulsions in humans. A previous report of a human poisoned with acetamiprid suggests that the consequences of the abnormal electrocardiogram changes in case 2 may be due to the active ingredient acetamiprid. The observed metabolic acidosis with a high anion gap could be due to the accumulation of lactic acid in cases 1 and 2, but it is more likely that metabolic acidosis was due to the DEG. However, the patient in case 2 excreted a normal amount of urine and showed no evidence of acute renal failure (normal blood urea nitrogen and creatinine). The symptoms of DEG were not severe in case 2, despite the high concentration of DEG. This may have been due to her severe vomiting. The co-formulants do not explain the hypokalemia. Additional studies are required before a characteristic toxic presentation of acetamiprid can be defined. The symptoms we observed in this study are similar to acute OP, including convulsion (a central nervous system effect), hypotension (a muscarinic receptor effect) and muscle weakness (a nicotinic receptor effect). However, the main muscarinic signs of acute OP poisoning, including miosis mucous supersecretion, excessive sweating, bradycardia, epiphora, diarrhoea, were not observed, neither was the serum cholinesterase level depressed. We performed treatment with gastric lavage, activated charcoal and cathartics in the present patients, because we

	were not familiar with acetamiprid poisoning. A gastric lavage was also done 2 h after ingestion in the other Japanese case. It is therefore uncertain that these treatments are appropriate to treat patients poisoned with this chemical. Retrospectively, it appeared that a gastric lavage was unnecessary on the basis of the clinical manifestations by the acetamiprid poisoning and the duration after the ingestion, especially in case 1. Acetamiprid appears to have a low toxicity in mammals, as indicated in animal experiments, but it is possible that the ingestion of larger amounts may lead to serious conditions that require additional supportive care.
Overall assessment	Reliable first hand reporting from a clinical setting of acute acetamiprid poisoning in humans. Essential was the knowledge of the co-formulants, allowing these potentially confounding elements to be teased-out.

Acute poisoning with neonicotinoid insecticides: A case report and literature review	
KCA 5.9.3; KCA 5.9.5; KCA 5.9.6	
Author(s)	Lin, P-C., Lin, H-J., Liao, Y-Y., Guo, H-R., Chen, K-T.
Year	2013
Journal	Basic & Clinical Pharmacology & Toxicology, 2013, 112, 282–286
Relevance check	Relevant
Reliability check	1
Reasons for no reliability	
Summary	Neonicotinoids are a new class of insecticides widely applied for crop protection. These insecticides act as agonists at nicotinic acetylcholine receptors, which cause insect paralysis and death. The high specificity for receptors in insects was considered to possess highly selective toxicity to insects and relative sparing of mammals. However, an increasing number of cases of acute neonicotinoid poisoning have been reported in recent years. We reported a man who developed respiratory failure and shock after ingestion of a neonicotinoid insecticide. A detailed literature review found that respiratory, cardiovascular and certain neurological presentations are warning signs of severe neonicotinoid intoxication. The amounts of ingested neonicotinoid insecticide and the plasma neonicotinoid concentration are not useful guides for the management of intoxicated patients. Supportive treatment and decontamination are the practical methods for the management of all neonicotinoid-poisoned patients.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	None. Case study. First hand reporting of patient poisoned with formulation containing imidacloprid. Other materials in the formulation are also known, allowing any known co- symptoms of poisoning with the co-formulants to be accounted-for.

Test substance Identification of test substance, source, purity, stability	A formulation ('Tie-Sha-Zhang'), containing 9.6% imidacloprid in N-methyl-2-pyrrolidone.
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	A 56-year-old man with a history of depression was treated in a psychiatric clinic regularly for 20 years. His wife found him attempting to commit suicide by ingesting approximately 40 mL of pesticide 20 min. before his arrival at the hospital emergency department.
Controls Positive control, negative	None
Dosing system Exposure (dose, duration, frequency)	Single self-administered oral dose of 40 mL of formulation.
Statistical analyses Sample size/replicates statistical analysis of data (significance level, variability)	None
Results Determined effect concentration, dose response observed	At the time of arrival in the emergency department of the hospital, his vital signs were as follows: body temperature, 36°C; pulse, 79 beats/min.; respiratory rate, 24/min.; and blood pressure, 87/ 56 mmHg. A physical examination revealed a drowsy man with dyspnoea, diaphoresis, drooling and multiple oral ulcers. Laboratory investigations demonstrated a white cell count of 13,900/µL, severe lactic acidosis (lactate 9.5 m/mole), 17 mg/dl blood urea nitrogen and 1.6 mg/dl creatinine. A plain radiograph of the chest was normal and an electrocardiogram showed sinus tachycardia. The patient was intubated with mechanical ventilation 8 hr later for fever, persistent hypotension, profound dyspnoea and coma and was transferred to the intensive care unit. He underwent supportive care and treatment with intravenous antibiotics. The patient made an uneventful recovery. He was extubated on the eighth day of admission and was discharged 4 days later. **Discussion** A further 16 cases of human neonicotinoid poisoning (13 imidacloprid and 3 acetamiprid) are reviewed in the paper. The solvents used in neonicotinoids may play a role in the toxidromes of neonicotinoid poisoning. Many neonicotinoids use N-methyl pyrrolidone (NMP) as the solvent in Taiwan and Sri Lanka (the cases from the two countries constitute 93% of all reviewed cases). A large

	amount of NMP ingestion irritates the upper gastrointestinal tract and results in oral ulcers, nausea, vomiting, dysphagia, odynophagia and abdominal pain. In addition, airborne exposure to NMP induces central nervous system depression in rats, which may worsen the neurological symptoms of neonicotinoid intoxication. In neonicotinoid-intoxicated patients, neurological depression decreases airway protection and cardiac depression further aggravates the load of respiration. Furthermore, corrosive injury to the upper gastrointestinal tract induces mucosal oedema of the airway and the resultant inflammatory process precipitates fever and hypotension. The combination of aspiration due to the lack of airway protection, airway obstruction due to corrosive injury to the airway mucosa and increased respiratory load due to inflammation and shock contributes to the evolution of respiratory failure. Several mechanisms participate in the development of toxidromes in neonicotinoid-poisoned patients and the clinical features therefore varied between reports. Accordingly, physicians need to know the warning signs of severe neonicotinoid intoxication. We found that gastrointestinal symptoms and minor neurological presentations occurred equally in both the severe and non-severe groups. Conversely, respiratory, cardiovascular and some neurological symptoms (coma and mydriasis) occurred more commonly in severely intoxicated patients. Meticulous observation is indicated in neonicotinoid-poisoned patients presenting with these warning signs. The more severe cases had a greater tendency to result from oral ingestion than inhalation or dermal contact. However, the difference in the incidence of severe intoxication between the two routes of exposure is insignificant. Phau *et al*. found that the amounts of ingestion in severe poisoning cases were less than the amount in non-severe cases. The study from Sri Lanka discovered that the plasma concentrations of neonicotinoids were high and remained elevated up to 10–15 hr after ingestion in many intoxicated patients; however, only two patients developed severe symptoms. A report of two fatalities due to neonicotinoid intoxication in Portugal measured the post-mortem plasma concentrations, which were not greater than the median level demonstrated in the study from Sri Lanka. These facts revealed that the plasma concentration does not appear to be useful for guiding clinical management. The presence of severe respiratory, cardiovascular and neurological symptoms is a practical guide for treating patients with neonicotinoid poisoning **Recommendations for treatment** All poisoning patients should undergo skin decontamination and remove the contaminated clothes because neonicotinoids could be absorbed by inhalation and dermal contact. Gastrointestinal decontamination can be conducted by the insertion of a gastric tube

	and stomach content withdrawal. Gastric lavage and activated charcoal should be avoided whenever corrosive injuries to the oral and gastrointestinal mucosa are discovered. The severity of poisoning is not proportional to the plasma neonicotinoid concentration. Therefore, there is currently no role for haemoperfusion to increase neonicotinoid elimination. Supportive management is adequate for all neonicotinoid-poisoned patients. **Conclusions** Respiratory, cardiovascular and certain neurological presentations (dyspnoea/apnoea, coma, tachycardia, hypotension, mydriasis and bradycardia) are warning signs of severe neonicotinoid intoxication. The amounts of ingested neonicotinoid and the plasma neonicotinoid concentration are not useful guides for the management of intoxicated patients. Supportive treatment and decontamination are the current practical management methods for all neonicotinoid poisoned patients.
Overall assessment	Reliable first hand reporting from a clinical case of acute imidacloprid poisoning in humans, and examination of additional cases of poisoning with neonicotinoids, including acetamiprid. Essential was the knowledge of the co-formulants, allowing these potentially confounding elements to be teased-out.

Oxidative stress: Role in acetamiprid-induced impairment of the male mice reproductive system	
KCA 5.6	
Author(s)	Zhang, J-J., Wang, Y., Xiang, H-Y., Li, M-X., Ma, K-G., Wang, X-Z., Zhang, J-H.
Year	2011
Journal	Agricultural Sciences in China Vol. 10(5), pp. 786-796
Relevance check	Relevant
Reliability check	2
Reasons for no reliability	Not applicable
Summary	The objective of this study was to examine the effect of acetamiprid on the reproductive function of male mice and to study the role of oxidative stress in acetamiprid-induced damage to the testes. Fifty adult Kunmin male mice (25-30 g) were divided into five groups (n=10 per group), i.e., control, blank, acetamiprid alone, acetamiprid and vitamin E and vitamin E alone. All groups were treated for 35 d. The results showed that acetamiprid significantly decreased the body weight and the weight of testosterone responsive organs, such as the testis, epididymis, seminal vesicle and prostate. Furthermore, acetamiprid also significantly reduced the serum testosterone concentration and decreased sperm count, viability, motility and the intactness of the acrosome ($P<0.05$ for each parameter). The mice treated with acetamiprid had damaged seminiferous tubules and

	Leydig cells based on the histological structure of testes; there was degeneration of the mitochondria and endoplasmic reticulum of Leydig cells. These deleterious effects of acetamiprid may be mediated by increasing oxidative stress, as acetamiprid increased malondialdehyde and nitric oxide in the testes, reduced the activity of catalase, glutathione peroxidase, superoxide dismutase and activated p38. The concentration of acetamiprid in the testes was lower than that in liver. Liver function tests, including aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP), suggest that male reproductive function may be affected through the indirect action of its metabolites. Vitamin E significantly ameliorated the effects of acetamiprid. We conclude that acetamiprid damages male reproductive function through inducing oxidative stress in the testes.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	None Not GLP
Test substance Identification of test substance, source, purity, stability	Acetamiprid (>97% pure, Shanghai Yongyuan Chem. Ltd.)
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	Kunmin male mice weighing 25-30 g were supplied by the Chonging Academy of Chinese Materials Medica. The animals were housed in rooms under a controlled temperature (22±2°C) with 50-60% relative humidity and a 12 h L/12 h D photoperiod, with *ad libitum* access to water and food pellets. The animals were acclimatized to laboratory conditions for up to 7 d prior to the gavages. According to a preliminary test, the dose of acetamiprid was 30 mg/kg bw. Fifty male mice were randomly allocated into five groups (n=10 per group). The groups were as follows: (I) control; (II) blank (peanut oil); (III) 30 mg/kg acetamiprid; (IV) 30 mg/kg acetamiprid + 20 mg/kg vitamin E; and (V) 20 mg/kg vitamin E. Both acetamiprid and vitamin E were dissolved in 0.1 mL peanut oil and delivered orally every day for 35 d. At 36 d after the start of treatment, all mice were sacrificed. **Haematological biochemical analysis and hormone assay** Blood samples were taken from the eye sockets of mice under anaesthesia using a 1 mL syringe before they were sacrificed. Blood samples were centrifuged at 5,000 r/min for 4 min and the serum samples were stored at 4°C for haematological biochemical analysis or -70°C until hormone analyses were performed. The activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were determined using an automatic chemistry analyser. Serum hormone concentrations were assayed using ELISA, according to the kit instructions. The sensitivity was

	0.1 ng/mL. **Sperm collection and analysis of sperm output** The excised left epididymis was weighed and the sperm collected. The sperm were collected by centrifugation with saline-merthiolate-triton (SMT). The number of sperm was measured using a hemocytometer Epididymal sperm counting results were expressed as the number of sperms per gram of epididymis. 100 sperm from each epididymis were assayed for viability and malformations. Sperm viability was assessed by the eosin Y stain and the motility of sperm was assayed by the number of sperm that could move in a line. The percentage of viable sperm and the motility of sperm were calculated. The rate of sperm malformation was assayed by the motility of sperm and whether the acrosome was intact. The integrity of the acrosome was assessed using the Wright-Giemsa stain. **Ultrastructure of the Leydig cells and histological structure of the testis and epididymis** Samples of testis and epididymis were immersion-fixed in Bouin's solution for histopathology and embedded in paraffin. Serial sections (5 μm thick) were cut and stained with haematoxylin and eosin (H&E). The sections were mounted with dextran plasticizer xylene (DPX) and examined using Leica light microscopy. Leydig cells were quantified in the interstitium between seminiferous tubules stained by H&E. The ultrastructure of Leydig cells were analysed. Samples of testes were cut into 2-mm-thick slices and fixed in ice-cold fixative consisting of 4% paraformaldehyde, 0.25% glutaraldehyde and 0.15 mol/L Hepes-KOH buffer (pH 7.4) for 30 min. Samples were post-fixed in 2% osmium tetroxide, dehydrated and embedded in Araldite 502. Ultra-thin sections (70-90 nm thick) of the blocks were picked up on copper grids, sections were stained with uranyl acetate and lead citrate and analysed under transmission electron microscope (TEM) at 80 kV. **Antioxidant enzyme activities and oxidative stress assays** Homogenization procedure of testes tissue was carried out for 2 min at 12 861×g in 5 mL of ice-cold Tris-HCl buffer (0.01 mol/, pH 7.4) containing 0.01% EDTA-2Na, 0.01 mol/L saccharose and 0.8% NaCl. All procedures were performed at 4°C. Homogenate, supernatant and extracted samples were prepared to determine the activities of CAT, GSH-Px, T-SOD, malondialdehyde (MDA) and NO. **Western blot analysis** Protein was isolated from testicular tissue using SDS PAGE and Western blot analyses. After blocking in PBS that contained 2%

	Tween-20 and 3% bovine serum antigen (BSA), membranes were incubated in a 1:200 dilution of primary antibody (anti-p38) and in a 1:1000 (antiphospho-p38) dilution of primary antibody in 5% phosphate-buffered saline-Tris (PBST) at 4°C overnight. The membranes were washed three times and then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2500) at room temperature for 1 h. Reactive bands were visualized by SuperSignal® West Pico chemiluminescent substrate and the membranes were then subjected to X-ray autoradiography. The Western blot X-ray films were scanned using Uniscan A688 and Smart Panel Scan software. Band intensities were determined by Quantity One software. The densitometry value of the phospho-p38 (p-p38) signal was divided by the value of the total p38 in the same lane in order to normalize the value to the protein load. **Total residues of acetamiprid in testes and livers** Testes or liver tissues (0.2 g) were ultrasonically extracted for 1 h with anhydrous sodium carbonate (1 g) and diamine methane (5 mL) using a homogenizer and centrifuged for 5 min at 12 861×g. The sample was dried before acetonitrile (20%) was added. Different concentrations of standard acetamiprid (2, 5, 10, 50 and 100 ng/mL) were used and detected in duplicate. The volume of each sample was 50 μL. High performance liquid chromatography (HPLC) was performed with a C18 column, methanol for the mobile phase A and 1% acetic acid water for the mobile phase B. The flow rate was 0.3 mL/min. Mass spectrometry (MS) conditions were: selected reaction monitoring (SEM) acquisition, the parent ion was 223.054, the qualitative ion was 73.054 m/z and the quantitative ion was 126.047 m/z. We took the mass concentration (ng/mL) for the horizontal and the peak area for the vertical coordinates, then drew the standard curve to enable the regression equation to be calculated. Tissue residues were calculated by the following formula: $$\text{Residues (ng g}^{-1}) = [(A \times C \times V)/As \times m] \times n$$ Where, A was the sample size, C was the standard preparation concentration, V was the volume size, n was the dilution multiple, as was the standard peak area and m was the sample quality.
Controls Positive control, negative	• Non gavage controls, • Vehicle controls (peanut oil only), • There were no positive controls, • Vitamin E controls.
Dosing system Exposure (dose, duration, frequency)	Gavage, daily at 35 mg/kg for 35 days. Groups were as follows: (I) control; (II) blank (peanut oil); (III) 30 mg/kg acetamiprid; (IV) 30 mg/kg acetamiprid + 20 mg/kg vitamin E; and (V) 20 mg/kg vitamin E.
Statistical analyses	Statistical analyses were performed using SPSS. All percentage data

Sample size/replicates statistical analysis of data (significance level, variability)	were subjected to arc-sine transformation before statistical analysis. Data were analysed by one-way ANOVA and the Fisher's least significant difference (LSD) method to determine treatment differences. A probability of $P<0.05$ was considered to be statistically significant.
Results Determined effect concentration, dose response observed	**Effect of acetamiprid on body weight gain and the weight of testis, epididymis, seminal vesicles and prostate gland** Compared to the controls, acetamiprid decreased body weight and the weight of the testis, epididymis, seminal vesicles and prostate gland ($P<0.05$). Vitamin E significantly ameliorated the effect of acetamiprid on body weight and testis, epididymis, seminal vesicles and prostate gland, compared to the acetamiprid only group ($P<0.05$). The body weight and testosterone-responsive organs were not affected in the blank (peanut oil) and vitamin E groups ($P>0.05$). **Acetamiprid negatively affected sperm output and quality** Compared to the control group, acetamiprid decreased sperm number, viability and motility ($P<0.05$), while increased the rate of acrosome deformity ($P<0.05$). Vitamin E reduced these adverse effects of acetamiprid by increasing sperm count, viability and sperm motility ($P<0.05$) and decreasing the rate of spermatic malformations ($P<0.05$). Compared to the control group, administration of peanut oil and vitamin E had no effect on sperm count, viability, motility and intact acrosome rate ($P>0.05$). **Effect of acetamiprid on serum testosterone concentration** Compared to the controls, serum testosterone level decreased in the acetamiprid only group ($P<0.05$). Vitamin E increased the concentration of testosterone compared to the acetamiprid only group. Peanut oil and vitamin E had no effect on testosterone concentration ($P>0.05$). **Effect of acetamiprid on histological structure of the testis and epididymis** Testes from the control group were in various stages of spermatogenesis; Leydig cells were abundant in the interstitium. In the acetamiprid only group, there was vacuolization of the seminiferous tubules and the number of spermatids and interstitial Leydig cells were obviously decreased. Moreover, some cells sloughed from the lumen of the seminiferous tubules, some primary spermatocytes vacuolized and the interstitium got widened. In the acetamiprid with vitamin E group, some spermatozoa remained within the seminiferous tubules, the number of spermatids and interstitial Leydig cells increased and the interstitial space was smaller in comparison to the acetamiprid only group. Peanut oil and vitamin E had no obvious effect compared to the controls. In the control group, sperms were numerous in the lumens of the epididymis. In the acetamiprid only group, there was almost no sperm

	in the lumens of the seminiferous tubules. Vitamin E increased the number of sperm in comparison to the acetamiprid group. Peanut oil and vitamin E had no effect on the epididymis. **Effects of acetamiprid on the ultrastructure of Leydig cells** In the control group, Leydig cells had normal endoplasmic reticulum (ER) and mitochondrial profiles, the cytoplasmic organelles were abundant, chromatin distribution was normal and the structure of chromatospherite and the boundary of the nuclear membrane were clear. In the acetamiprid only group, a large number of mitochondria were swollen. Vitamin E appeared to prevent these structural changes to some degree. Organelles were abundant and structure of mitochondria was normal, but the chromatin was slightly aggregated. Furthermore, the structure of local endoplasmic reticulum was unclear when compared to the control. The administration of peanut oil and vitamin E had no effect on the ultrastructure of Leydig cells. **Effect of acetamiprid on oxidative stress** Acetamiprid increased MDA and NO concentrations compared to the controls ($P<0.05$). Vitamin E ameliorated the effect of acetamiprid and MDA and NO concentrations were lower in acetamiprid group that received vitamin E than in acetamiprid only group. Compared to the controls, peanut oil and vitamin E had no effect on the concentrations of MDA and NO. **Acetamiprid decreased the activity of antioxidant enzymes** In the acetamiprid group, the activity of CAT, GSH-Px and T-SOD was reduced compared to the control ($P<0.05$). Compared to acetamiprid only group, vitamin E increased the concentrations of CAT, GSH-Px and T-SOD ($P<0.05$). Compared to the control, peanut oil and vitamin E had no effect on antioxidant enzymes ($P>0.05$). **Effect of acetamiprid on p38 activity** Compared to the controls, the concentration of antiphospho-p38 protein was elevated with acetamiprid treatment and vitamin E prevented this elevation. Peanut oil and vitamin E had no effect on p38 activity. **Effect of acetamiprid on serum enzymes** Compared to the controls, acetamiprid increased the activity of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) ($P<0.05$). Vitamin E prevented this increase of these serum enzymes in comparison to acetamiprid only group. Peanut oil and vitamin E had no effect on the activity of ALT, AST and ALP ($P>0.05$). **Acetamiprid residue in the testes and livers** Acetamiprid was not detected in the testes and liver of the control,

	peanut oil and vitamin E groups. Compared to the controls, the amount of acetamiprid residue in the testes was increased in acetamiprid only group ($P<0.05$). Additionally, the concentration of acetamiprid in the liver was higher than that in the testes ($P<0.05$). Vitamin E reduced the amount of acetamiprid residue in the liver and testes in comparison to acetamiprid only group ($P<0.05$).
Overall assessment	The methods in this *in vivo* gavage study are well described and may indicate that effects on male reproductive function may be due to oxidative stress rather than any pharmacodynamics response. The data does not supersede existing data however and does not impact on the overall risk assessment for acetamiprid (the NOAELs in gavage studies in the existing data are lower that the dose used).

Nephrotoxicity of acetamiprid on male mice and the rescue role of Vitamin E	
KCA 5.3	
Author(s)	Zhang, J-J, Wang, Y., Xiang, H-Y., Zhang, J-H., Wang, X-Z.
Year	2012
Journal	Journal of Animal and Veterinary Advances Vol. 11(15), pp. 2721-2726
Relevance check	Relevant
Reliability check	2
Reasons for no reliability	Not applicable
Summary	The objective of this study was to examine the effect of acetamiprid on kidney of male mice and to study the ameliorative role of antioxidant on the nephrotoxicity of acetamiprid. Fifty adult Kunmin male mice (25-30 g) were divided into five groups (n = 10 per group): controls, blanks, acetamiprid alone, acetamiprid and vitamin E and vitamin E alone. All groups were treated for 35 days. The results showed that acetamiprid significantly increased the activity of urea, Cr and the concentration of P and decreased the concentrations of UA and Ca. The mice treated with acetamiprid had damaged renal corpuscles and tubules based on the histological structure of kidney. Furthermore, the acetamiprid residue in kidneys was lower than that in livers which suggests that renal function may be affected through the indirect action of acetamiprid metabolites. Vitamin E significantly ameliorated the effects of acetamiprid. Researchers conclude that acetamiprid could damage kidney which may be induced by the oxidative stress of acetamiprid metabolites. As an antioxidant, vitamin E can reduce the nephrotoxicity of acetamiprid.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	None Not GLP
Test substance Identification of test	Acetamiprid (>97% pure, Shanghai Yongyuan Chem. Ltd.)

substance, source, purity, stability	
Test system characterization and study design Description of the test system, source/origin of test system, information on conditions and maintenance, study protocol	Kunmin male mice weighing 25-30g were supplied by the Chonging Academy of Chinese Materials Medica. The dose of acetamiprid was 30 mg/kg bw. Fifty male mice were randomly allocated into five groups (n=10 per group). The groups were as follows: (I) control; (II) blank (peanut oil); (III) 30 mg/kg acetamiprid; (IV) 30 mg/kg acetamiprid + 20 mg/kg vitamin E; and (V) 20 mg/kg vitamin E. Both acetamiprid and vitamin E were dissolved in 0.1 mL peanut oil and delivered orally every day for 35 d. At 36 d after the start of treatment, all mice were anesthetized with halothane and killed aseptically by severing the neck vessels. **Haematological biochemical analysis** Blood samples were taken from the eye sockets of mice under anaesthesia using a 1 mL syringe before they were sacrificed. Blood samples were centrifuged at 5000 r/min for 4 min, and the serum samples were stored at 4°C for haematological biochemical analysis. The activities of urea, uric acid (UA), creatinine (Cr) and the concentrations of Calcium (Ca), Phosphorus (P) were detected according to the method described by Manna et al (2004) using an automatic Chemistry Analyser. **Histological structure of the kidney** Samples of kidney were immersion-fixed in Bouin's solution for histopathology and embedded in paraffin. Serial sections (5 µm thick) were cut and stained with Haematoxylin and Eosin (H&E) in the same fashion as in Zhang et al., 2011). **Total residues of acetamiprid in kidneys** Kidneys tissues (0.2 g) were ultrasonically extracted and samples were analysed by LC-MS/MS.
Controls Positive control, negative	• Non gavage controls, • Vehicle controls (peanut oil only), • There were no positive controls, • Vitamin E controls.
Dosing system Exposure (dose, duration, frequency)	Gavage, daily at 35 mg/kg for 35 days. Groups were as follows: (I) control; (II) blank (peanut oil); (III) 30 mg/kg acetamiprid; (IV) 30 mg/kg acetamiprid + 20 mg/kg vitamin E; and (V) 20 mg/kg vitamin E.
Statistical analyses Sample size/replicates statistical analysis of data (significance level, variability)	Statistical analyses were performed using SPSS (Version 16.0). Data were analysed by one-way ANOVA and Fisher's Least Significant Difference (LSD) Method to determine treatment differences. A probability of $P<0.05$ was considered to be statistically significant.
Results Determined effect concentration, dose	**Effect of acetamiprid on histological structure of the kidneys** In the control group, the structure of glomeruli was clear, capsular spaces were small, the boundaries of the visceral layer and parietal

response observed	layer of renal capsule were clear, the structure of epithelial cells in proximal convoluted tubules and distal convoluted tubules was normal. In the acetamiprid group, the glomeruli were atrophied and disintegrated, capsular spaces got widened obviously, the visceral layer and parietal layer of renal capsule were destroyed and some of them had disappeared, the epithelial cells in proximal convoluted tubules and distal convoluted tubules were swollen and some epithelial cells had vacuolization, the structure of epithelial cells was unclear, there were some cell fragments in the tubules. In the acetamiprid with vitamin E group, the atrophy degree of glomeruli was decreased and the capsular spaces were smaller in comparison to the acetamiprid only group, the boundaries of visceral layer and parietal layer of renal capsule were clear, the epithelial cells in proximal convoluted and distal convoluted tubules were slightly swollen, cell fragments were visible in some tubules. Compared to the controls, peanut oil and vitamin E had no obvious effect on the structure of kidney. **Effect of acetamiprid on haematological biochemical indicators of kidney** Compared to the controls, acetamiprid increased the activity of urea and Creatinine (Cr) by 111.11 and 25.28%, respectively and decreased the activity of Uric Acid (UA) by 33.02% ($p<0.05$ for all). Vitamin E weakened the effect of acetamiprid, the concentrations of urea and Cr were lower in the acetamiprid group that received vitamin E than in the acetamiprid only group which were decreased by 38.76 and 11.47%, respectively. Vitamin E increased the concentration of UA by 29.57%. Compared to the controls, peanut oil and vitamin E had no effect on the activity of urea, UA and Cr ($p>0.05$ for all). **Effect of acetamiprid on ion concentrations in the blood** Compared to the controls, acetamiprid increased the concentration of Phosphor (P) and decreased the concentration of Calcium (Ca) ($p<0.05$ for both). Vitamin E ameliorated the effect of acetamiprid, the concentration of P were lower in the acetamiprid group that received vitamin E than in the acetamiprid only group and the concentration of Ca was increased in comparison to the acetamiprid only group ($p>0.05$ for both). Peanut oil and vitamin E had no effect on the concentrations of P and Ca ($p>0.05$ for both). **Acetamiprid residue in the kidneys** Acetamiprid could not be detected in the kidney of the control, blank and vitamin E groups. Compared to the controls, the amount of acetamiprid residue in the kidney was increased in the acetamiprid only group which was 112.48 ng/g ($p<0.05$). Vitamin E reduced the concentration of acetamiprid residue in comparison to the

	acetamiprid only group which was decreased by 48.02% ($p<0.05$). The concentration of acetamiprid residue was indicated by grey area while the blank area showed no residue, the expected retention time was at 3.30 min, the relative intensity was compared to the kidney of chicken. In this study, acetamiprid significantly increased the activity of urea, Cr and the concentration of P and decreased the concentrations of UA and Ca. Acetamiprid damaged the structures of renal corpuscles and tubules, seriously affected renal function. In addition, the concentration of acetamiprid residue in kidneys was lower than that in livers which inferred that kidney might be affected through the indirect action of acetamiprid metabolites. Furthermore, the antioxidant vitamin E, ameliorated the deleterious effects of acetamiprid on kidney which indicated that acetamiprid might damage the kidney through the oxidative stress of metabolites and vitamin E could reduce the nephrotoxicity of acetamiprid. Acetamiprid mainly affect the nervous system of insects through the excessive activation of acetylcholine receptor. In addition to neurotoxicity, plasma cholesterol was significantly reduced and liver toxicity and gastrointestinal irritation has been shown in mice and mammals, respectively. The previous experiment showed that acetamiprid damaged male reproductive function through inducing oxidative stress in the testes of mouse (Zhang et al., 2011). To the knowledge, there is a paucity of reports with regards to whether acetamiprid effects on kidney. This novel study found that acetamiprid damaged the structure of kidney and affected the biochemical indicators of kidney which indicated that acetamiprid did affect the renal function. Urea and Cr are the indicators of renal function. Urea synthesis in the liver is the major final product of nitrogenous compounds metabolism in mammals. Creatinine forms from creatine phosphate in muscle through a spontaneous and irreversible way. Once renal function was impaired, the normal excretion of urea and creatinine were hampered by increasing the levels of urea and creatinine in serum. In addition, Calcium and Phosphorus are also indicators of renal function. Some studies have shown that the impairment of kidney function can reduce the activity of Vitamin D, inhibit the activity of l α-hydroxylase and the secretion of parathormone resulting in the concentration of serum calcium decreased and phosphorus increased. This study found that acetamiprid increased the concentrations of urea, Cr and P while the concentration of Ca was decreased which inferred that acetamiprid could damage the renal function. Uric acid can not only play a preventive anti-oxidation function through combination with iron and copper ions but also remove singlet oxygen and hydroxyl radicals directly. Recent studies have formed that Uric Acid (UA) was the highest content of antioxidants in the body and the content of UA in serum is an important parameter

	which represents the anti-oxidation capacity in body. This study found that acetamiprid decreased the activity of UA. Reduction of the levels of uric acid could lead to the relative increase of ROS which would inevitably lead to an increase in the amount of NO synthesis. NO was involved in regulating renal hemodynamics and inhibition of NO enabled the glomerular afferent arteriolar constrict, Renal Plasma Flow (RPF) and Glomerular Filtration Rate (GFR) decline. Some studies found that NO could directly affect vascular smooth muscle to reduce vascular tone and inhibit Tubuloglomerular Feedback (TGF). Acetamiprid injected *in vivo* could form eight metabolites which were regarded as agonists of nicotine and induced Nitric Oxide Synthase (iNOS) in mice. In this study, acetamiprid also damaged the structure of renal corpuscles and tubules consistent with the previous results which inferred that NO could be involved in acetamiprid-induced impairment of kidney in mice. The fact that vitamin E weakened the deleterious effects of acetamiprid on kidney provided new evidence that acetamiprid might damage kidney by inducing ROS. In order to provide evidence on whether the effects of acetamiprid on renal function are direct or indirect, researchers assessed the acetamiprid residue in the kidney. The results showed that the concentration of acetamiprid in kidneys was lower than that in livers and the previous experiment has found that acetamiprid increased the levels of AST, ALT and ALP (Zhang *et al.*, 2011) which gave further evidence that the detrimental effects of acetamiprid on the kidney were mediated by its metabolites. **Conclusions** In this study, acetamiprid has deleterious effects on kidney, potentially through the oxidative stress of its metabolites and vitamin E could reduce the nephrotoxicity of acetamiprid. Thus, acetamiprid should be used in a restricted and careful manner to protect mammalian renal capabilities.
Overall assessment	The methods in this in vivo gavage study are well described, and may indicate that effects on kidney function may be due to oxidative stress rather than any pharmacodynamics response. The data does not supersede existing data however, and does not impact on the overall risk assessment for acetamiprid (the NOAELs in gavage studies in the existing data are lower that the dose used).

CA 9.2.3 Ecotoxicology

Pesticide compatibility with natural enemies for pest management in greenhouse Gerbera daisies	
KCA 8.3.2	
Author(s)	Abraham, C.M., Braman, S.K., Oetting, R.D., Hinkle, N.C.
Year	2013

Journal	J. Econ. Entomol. Vol. 106(4), pp. 1590-1601
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Acetamiprid and eight other pesticides were individually evaluated in vial swirl assays for toxicity to a biological control agent, the predatory mite (*Neoseiulus californicus* [McGregor]). *N. californicus* were exposed for 12, 24 and 48 h at the median label rate. Acetamiprid was found to be moderately harmful (80-98% mortality) to harmful (causing >99% mortality) to *N. californicus* after exposure for 48 h.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Vial assay methods (Bjorksten and Robinson, 2005; Wu and Miyata, 2005) were modified and used as pesticide swirl assays for predatory mites.
Test substance Identification of test substance, source, purity, stability	Acetamiprid
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 22-25^{o}C • Photoperiod: 14:10 h light: dark cycle • Water: freshwater • Number of animals: 10 adults • Food availability: Drop of honey streaked inside each vial
Controls Positive control, negative control	Negative control: water control
Dosing system Exposure (dose, duration, frequency)	• Dose: Median label rate, 10-15 ml of designated treatment/glass vial • Duration: 48 h • Three trials
Test species Body weight or length, gender, age/life stage, source	• Predatory mite (*Neoseiulus californicus* [McGregor]) • Life stage: adult • Source: not specified
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	• Experiment unit: glass vial • Replicates: 10 • Treatments: 10 • Trials: 3 • Statistical analysis: ANOVA using the general linear model procedure, means were separated using Tukey's honestly

	significant difference test. • Tiered IOBC method used; considers pesticides from laboratory studies causing mortality rates of >99% harmful, 80-98% moderately harmful, 30-79% slightly harmful and <30% mortality harmless (Stark et al. 2007).
Biological effects Determined effect concentration, dose response observed	Mortality was investigated and acetamiprid was found to be moderately harmful (80-98% mortality) to harmful (causing >99% mortality) at the median label rate.
Overall assessment	Study provides information on the toxicity of acetamiprid to *N. californicus* and is considered to be of limited reliability as no analytical verification of the test item was performed and no information was provided on test substance purity or source. In addition, the study was not performed to a recognised guideline.

Toxicity of some commonly used insecticides against *Coccinella undecimpunctata* (Coleoptera: Coccinellidae)	
KCA 8.3.2	
Author(s)	Ahmad, M., Rafiq, M., Arif, M.I., Sayyed, A.H.
Year	2011
Journal	Pakistan J. Zool. Vol. 43(6), pp. 1161-1165
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The effects of five insecticides including acetamiprid were tested for their residual effects on *Coccinella undecimpunctata* using a glass vial method and treated leaves. Mortality of adults at 24, 48 and 72 hours ranging for 50-91% and 10-78% was observed in glass vials and treated leaves, respectively. Acetamiprid was the least toxic insecticide in the residual film method but the most toxic in the glass vial method.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Treatment of insects (i) directly with field doses of insecticides in glass vials, (ii) insecticide-treated leaf discs in leaf dip method, (iii) with insecticide-treated leaves of field sprayed plots placed in glass vials and (iv) with serial concentrations of insecticides in glass vials in order to determine their LC_{50} values (Ahmad et al. 2008).
Test substance Identification of test substance, source, purity, stability	Acetamiprid (Mospilon® 125SP)
Test conditions Temperature, pH, oxygen concentration, water hardness,	• Temperature: 25 ± 2ºC • Relative humidity: 65 ± 5% • Photoperiod: 16:8 h light:dark cycle • Number of animals: 10 adults

<table>
<tr><td>conductivity, photoperiod, light intensity, number of animals, food availability</td><td>• Food availability: mealybug nymphs</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Control: untreated glass vials</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>• Dose: 125 mL/100 L
• Duration: 72 h
• Three replicates with 10 beetle per glass vial (n = 30)</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>• Eleven-spot ladybird beetle Coccinella undecimpunctata
• Life stage: adult
• Source: Cotton field at Central Cotton Research Institute (Multan, Pakistan)</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>• Experiment unit: Scintillation glass vials (30 ml)
• Replicates: 3
• Treatments: 10
• Statistical analysis: Mortality data was corrected using Abbott (1925) formula which was then used to determine LC_{50} values and their confidence interval using probit analysis (Finney, 1971) in POLO-PC. Mean comparisons were performed using the least significant difference (LSD).</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Percent mortality from exposure in glass vials
<table>
<tr><td></td><td colspan="3">% mortality</td></tr>
<tr><td></td><td>24 h</td><td>48 h</td><td>72 h</td></tr>
<tr><td>Acetamiprid</td><td>63.3</td><td>83.3</td><td>83.3</td></tr>
<tr><td>Control</td><td>0</td><td>0</td><td>0</td></tr>
</table>
Table 2: Percent mortality from exposure to leaves treated with leaf dip method
<table>
<tr><td></td><td colspan="3">% mortality</td></tr>
<tr><td></td><td>24 h</td><td>48 h</td><td>72 h</td></tr>
<tr><td>Acetamiprid</td><td>20</td><td>43.3</td><td>53.3</td></tr>
</table>
Table 3: Percent mortality from exposure to field sprayed leaves
<table>
<tr><td></td><td colspan="3">% mortality</td></tr>
<tr><td></td><td>24 h</td><td>48 h</td><td>72 h</td></tr>
<tr><td colspan="4">Leaves exposed 2 h after spray</td></tr>
<tr><td>Imidacloprid</td><td>6.7</td><td>26.7</td><td>23.3</td></tr>
<tr><td>Acetamiprid</td><td>43.3</td><td>63.3</td><td>73.3</td></tr>
<tr><td colspan="4">Leaves exposed 24 h after spray</td></tr>
<tr><td>Imidacloprid</td><td>10</td><td>10</td><td>13.3</td></tr>
<tr><td>Acetamiprid</td><td>16.7</td><td>16.7</td><td>16.7</td></tr>
</table></td></tr>
</table>

<table>
<tr><td></td><td>Imidacloprid and acetamiprid were compared for toxicity of field sprayed leaves with mortality decreasing from 23 to 13% with delay of 24 hour exposure for imidacloprid and 73 to 63% for acetamiprid. High mortality occurred with acetamiprid which was also toxic in laboratory treated leaf discs. Decline in mortality to 13% due to exposure to imidacloprid-treated leaves suggest that imidacloprid was the safest tested insecticides to C. undecimpunctata.

Table 4: Response of adults for laboratory bioassay for LC50 values
<table>
<tr><th></th><th>Time (h)</th><th>LC50 (µl/mL)</th></tr>
<tr><td rowspan="2">Acetamiprid</td><td>48</td><td>93.5</td></tr>
<tr><td>72</td><td>50.0</td></tr>
</table>
Data after 48 h exposure showed, however, acetamiprid as the least toxic insecticide compared with imidacloprid and pyrethroids tested. Acetamiprid was also least toxic after 72 h.</td></tr>
<tr><td>Overall assessment</td><td>The study provides information on the toxicity of acetamiprid to C. undecimpunctata and is considered of limited reliability because chemical analyses to verify the test concentrations were not described. In addition, the study was not performed to a recognised guideline.</td></tr>
</table>

Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior	
KCA 8.3.1	
Author(s)	Aliouane, Y., El Hassani, A.K., Gary, V., Armengaud, C., Lambin, M., Gauthier, M.
Year	2009
Journal	Environ. Toxicol. Chem. Vol. 28(1), pp. 113-122
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Laboratory bioassays were conducted to evaluate the effects on honeybee behaviour of sublethal doses of insecticides chronically administered orally or by contact. Emergent honeybees received a daily dose of insecticide ranging from one-fifth to one-five-hundredth of the median lethal dose (LD50) during 11 d. After exposure to fipronil, acetamiprid or thiamethoxam, behavioural functions of honeybees were tested on day 12. Fipronil (0.1 ng/bee), induced mortality of all honeybees after one week of treatment. As a result of contact treatment at 0.01 ng/bee, honeybees spent significantly more time immobile in an open-field apparatus and ingested significantly more water. In the olfactory conditioning paradigm, fipronil-treated honeybees failed to discriminate between a known and an unknown odorant. Thiamethoxam by contact induced either a significant

	decrease of olfactory memory 24 h after learning at 0.1 ng/bee or a significant impairment of learning performance with no effect on memory at 1 ng/bee. Responsiveness to antennal sucrose stimulation was significantly decreased for high sucrose concentrations in honeybees treated orally with thiamethoxam (1 ng/bee). The only significant effect of acetamiprid (administered orally, 0.1 µg/bee) was an increase in responsiveness to water. The insecticides tested have limited effects on the motor, sensory and cognitive functions of the honeybee.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Fipronil (98.5% purity), thiamethoxam (97% purity) and acetamiprid (99% purity)
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 33ºC • Relative humidity: 40% • Photoperiod: Maintained in darkness • Number of animals: 40 • Food availability: pollen and sucrose solution (50% w/v) were provided ad libitum for the first week; for the 11 d exposure bees were fed sucrose solution (50% w/v) and water changed daily
Controls Positive control, negative control	Control: For oral exposures, control groups ingested a sugar solution containing the appropriate solvent; for contact exposures solvent alone was applied
Dosing system Exposure (dose, duration, frequency)	• Dose: fipronil – 0.01 and 0.1 ng, thiamethoxam – 0.1 and 1.0 ng, acetamiprid 0 0.1 and 1.0 µg • For oral exposures: volume of sucrose solution adjusted daily on assumption 33 µl/bee/d consumed • For contact exposures: 1 µl of the solution was applied to the thorax using a micropipette with a tip • Duration: 11 d
Test species Body weight or length, gender, age/life stage, source	• *Apis mellifera* • Life stage: emergent • Source: Hives at Toulouse, in the south of France
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Experiment unit: Caged bees Sample size: 22 to 58 bees per experiment depending on test Comparison of the mortality curves between the control and treated groups was performed with the Kaplan–Meier test. For locomotor tests, student's *t* tests were performed for mean comparison between treated and control group values after variance comparison with

	Levene's test. Daily values of consumed water were compared between the treated and the control groups with a Student's *t* test. Responsiveness to water was compared between control and treated groups at 1 and 3 h with a chi-square test. The comparisons between the groups for sucrose responsiveness were conducted using Fisher's exact test, which directly yields a p value. For olfactory learning, the values were compared between control and treated groups for acquisition (from the second to the fifth trial) and for each retention test (at 1, 24 and 48 h) using Fisher's exact test. Within-group comparison for level response to conditioned odorant versus new odorant was performed using McNemar's test. For each of these tests, a *p* value of less than 0.050 was considered significant. All the statistical tests were performed with SPSS® 12 software.
Biological effects Determined effect concentration, dose response observed	Repeated exposure of honeybees to fipronil at the dose of 0.1 ng/bee induced complete mortality in individuals exposed for one week. This effect on mortality was not observed after neonicotinoid exposure. Sustained exposure to fipronil or to neonicotinoids induced limited behavioural modifications. Chronic sublethal doses of acetamiprid induced no greater effect than at acute doses on water responsiveness and induced less effect than at acute doses on locomotor activity, sucrose responsiveness and olfactory memory. The experiments with thiamethoxam show that repeated exposure to a dose that has no behavioural effect when applied in acute conditions results in the appearance of some behavioural deficits. We may conclude from these observations that acetamiprid seems to be the least toxic of the three molecules for honeybees after repeated exposure to sublethal doses. We reported a mean mortality level of 21% for acetone and 12% for acetonitrile in control animals orally or topically treated with the solvent. Comparison to the mortality level (10%) reported in nontreated animals for a 10-d observation period indicates that acetone enhances mortality of individuals in our experiments and can be considered partly responsible for the mortality of fipronil-treated (0.01 ng/bee) and acetamiprid-treated (1 µg/bee and 0.1 µg/bee) animals. Oral thiamethoxam delivered at the highest dose (one-fifth of the LD50 corresponding to 30 µg/L) had no significant effect on mortality. Similarly, chronic oral exposure of honeybees to either imidacloprid or its plant metabolites induced no lethal effect at concentrations of 20 and 40 µg/L. Acetamiprid 1 µg/bee induced the highest observed mortality level (30%), but this level was not statistically different from that of the control group. Oral fipronil at a dose of one-fiftieth of the (0.1 ng /bee, 3 µg/L) induced complete mortality after one week of treatment. We report limited effects of the three pesticides on the motor, sensory

	and cognitive functions of the honeybee. The behavioural functions we have taken into account are linked to the foraging profile of the honeybee. We observed modifications of behavioural response to water after treatment with pesticides. Fipronil induced an increase in water consumption during the exposure period. Oral acetamiprid treatment (0.1 µg/bee) induced the enhancement of water responsiveness and a nonsignificant increase of sucrose responsiveness was induced by topical acetamiprid (1 µg/bee). We report limited effects of the three pesticides on the motor, sensory and cognitive functions of the honeybee. The behavioural functions we have taken into account are linked to the dose of 1 ng/bee, but there were no significant repercussions on olfactory memory.
Overall assessment	Sublethal effects of pesticides on honeybees were investigated but the study is considered of limited reliability because chemical analyses to verify the test concentrations were not described. In addition, the study was not performed to a recognised guideline.

Field tests on vines and apple trees and in the laboratory	
KCA 8.3.2	
Author(s)	Baldessari, M., Malagnini, V., Tolotti, G., Angeli, G.
Year	2010
Journal	Informatore Agrario Vol. 66(45), pp. 67-70
Relevance check	Relevant
Reliability check	4
Reasons for no reliability	Due to the severe lack of details in methodology, statistical analysis and results, this article is not reliable.
Summary	The objectives of this research were to evaluate several pesticides in laboratory studies and field application trials to *Amblyseius andersoni*. Acetamiprid tested at two different doses on vines and apple trees did not show significant affects to *A. andersoni*. The laboratory tests showed acetamiprid did not cause significant mortality to *A. andersoni*.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No methods have been detailed
Test substance Identification of test substance, source, purity, stability	Acetamiprid, Epik, 20% a.s.
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light	No test conditions are reported

<table>
<tr><td>intensity, number of animals, food availability</td><td></td></tr>
<tr><td>Controls
Positive control, negative control</td><td>No information on the use of controls is provided.</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>No data is reported on the testing system for laboratory tests or how field studies were carried out.

Acetamiprid: 37.5 g/hL</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Predatory mite Amblyseius andersoni</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>No details are reported</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 3: Mortality levels of applications of acetamiprid on the apple tree and vines with different dosages and formulations
<table>
<tr><th rowspan="2">Crop</th><th rowspan="2">Year</th><th rowspan="2">% s.a.</th><th rowspan="2">Dose (g/hL)</th><th colspan="3">Mortality (%)*</th></tr>
<tr><th>T + 7 days</th><th>T + 14 days</th><th>T + 21 days</th></tr>
<tr><td>Apple tree</td><td>2006</td><td>20</td><td>25</td><td>32</td><td>18</td><td>0</td></tr>
<tr><td>Apple tree</td><td>2006</td><td>20</td><td>37.5</td><td>44</td><td>13</td><td>0</td></tr>
<tr><td>Apple tree</td><td>2007</td><td>5</td><td>100</td><td>30</td><td>10</td><td>0</td></tr>
<tr><td>Apple tree</td><td>2010</td><td>5</td><td>133.3</td><td>36</td><td>22</td><td>11</td></tr>
<tr><td>Vines</td><td>2008</td><td>5</td><td>100</td><td>15</td><td>0</td><td>0</td></tr>
</table>
*days from treatment in brackets (T).

No significant collateral effects were shown; acetamiprid fell within the selective (M) or slightly harmful (N) class; it also demonstrated good selectivity on vines.

Table 4: Evaluation in the laboratory of the selectivity of acetamiprid
<table>
<tr><th>Active substance</th><th>Mortality Abbott (%)*</th><th>Fertility (%)*</th><th>Toxicity (%) E*</th><th>Class IOBC</th></tr>
<tr><td>Acetamiprid</td><td>5.00</td><td>0.81</td><td>23.09</td><td>1</td></tr>
</table>
*Correct mortality with respect to the sample (Abbott); fertility index; toxicity level E.
The value of the class IOBC was assigned to each toxicity level E as: Class 1 = selective formula (< 30%); Class 2 = slightly harmful formula (30-79%); Class 3 = harmful formula (80-99%); Class 4 = very harmful formula (>99%).</td></tr>
<tr><td>Overall assessment</td><td>Effects of acetamiprid on A. andersoni were investigated but due to the severe lack of details in methodology, statistical analysis and</td></tr>
</table>

	results, this article is not considered to be reliable.

Toxicity of biopesticides and synthetic insecticides to egg parasitoid, *Trichogramma chilonis* Ishii and coccinellid predator, *Cheilomenes sexmaculata* (Fabricius)	
KCA 8.3.2	
Author(s)	Basappa, H.
Year	2007
Journal	J. Biol. Control. Vol. 21(1), pp. 31-36
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Six insecticides including acetamiprid and several biopesticides were investigated for their toxicity to the egg parasitoid, *Trichogramma chilonis* Ishii and the coccinellid predator, *Cheilomenes sexmaculata* (Fabricius) under laboratory conditions. Most of the biopesticides were found to be safe to *T. chilonis* and *C. sexmaculata*. Among the synthetic insecticides, carbosulfan was found to be highly toxic to both species followed by acetamiprid.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Acetamiprid
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 27 ± 1°C • Relative humidity: 60 ± 5% • Photoperiod: Not described • Number of animals: 15 adults • Food availability: aphids and cowpea sprouts
Controls Positive control, negative control	Negative control: distilled water control
Dosing system Exposure (dose, duration, frequency)	• Dose: Acetamiprid (0.002%) • Duration: 72 h
Test species Body weight or length, gender, age/life stage, source	• Egg parasitoid (*Trichogramma chilonsis* Ishii) – use of parasitised eggs • Coccinellid predator (*Cheilomenes sexmaculata* [Fabricius]) - adults

	• Source: Parasitised eggs and adults reared in the Entomology laboratory, Directorate of Oilseeds Research, Rajendranagar, Hyderabad.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	• Experiment unit: 20 x 1.5 cm glass tube • Replicates: 3 • Treatments: 15 adults per treatment • Statistical analysis: data subjected to angular transformation and statistically analysed, analyses not specified
Biological effects Determined effect concentration, dose response observed	Effects of acetamiprid to on emergence of *T. chilonsis* adults was 16%, 11.33% and 18.66% on 1 day, 3 day and 7 day old parasitised eggs, respectively. Mortality from acetamiprid to *C. sexmaculata* was 75.5% after 24 h and 100% after 48 h.
Overall assessment	This study investigates the toxicity of a number of insecticides on *T. chilonis* and *C. sexmaculata* but is considered to be of limited reliability as no analytical verification of the test item was performed, statistical tests not defined and no information was provided on test substance purity or source. In addition, the study was not performed to a recognised guideline.

Impacts of orchard pesticides on *Galendromus occidentalis*: Lethal and sublethal effects	
KCA 8.3.2	
Author(s)	Beers, E.H., Schmidt. R.A
Year	2014
Journal	J. Econ. Entomol. Vol. 106(4), pp. 1590-1601
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Fifteen pesticides were tested in laboratory bioassays on *Galendromus occidentalis* (Nesbitt), the principal phytoseiid mite predator in Washington apple orchards. In female bioassays, acetamiprid mortality was moderate and all treatments caused higher rates of mortality than the control but were not different from each other. The 2x dose caused a 10-fold reduction in the number of total prey consumer with a 5.6-fold reduction in the 0.1x dose. The reduction in fecundity was similar in magnitude to the reduction in prey consumption at the two higher doses. No live larvae were produced in the 2x dose and numbers were significantly reduced in the 1x and 0.1 x doses although all eggs hatched. In larvae bioassays, acetamiprid was moderately toxic with one or more doses significantly higher than the control.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines	No protocol cited, but detailed methods are reported.

(US EPA, OECD, …)	
Test substance Identification of test substance, source, purity, stability	Acetamiprid, Assail 70 WP, 700 g/kg
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 20 ± 2°C • Photoperiod: 16:8 h light:dark cycle • Number of animals: 1 adult/disc (female bioassay); 20 females allowed to oviposit (larval bioassay) • Food availability: Spider mite *Tetranychus urticae* eggs
Controls Positive control, negative control	Negative control: distilled water control
Dosing system Exposure (dose, duration, frequency)	Acetamiprid was applied at 179 mg a.s./L as the 1x concentration and also tested at 2x (358 mg a.s./L) and 0.1x (18 mg a.s./L) doses. The test materials were applied with a Potter Spray Tower set at 44.8 kPa was used with intermediate nozzle. Each arena with a leaf disc was sprayed with 2 mL of the appropriate concentration (17.7 µL solution/cm^2 leaf area). Experimental unit: bean leaf disc cut from untreated, uninfested bean leaf and placed with the lower surface facing up in a plastic cup filled with cotton and water. A smaller (2.2 cm diameter) disc was used for single female organisms and a larger disc (3.5 cm diameter) was used for multiple individuals (larval bioassays). For the female bioassay, treatments were applied by contact to the *G. occidentalis* females and *T. urticae* eggs on the discs. After 24 and 48 h, live and dead females were counted, with discs held for 3 to 4 days to allow eggs to hatch. For larval bioassay: discs with eggs and larvae were sprayed in a Potter Spray Tower and mortality evaluated after 48 h
Test species Body weight or length, gender, age/life stage, source	• Phytoseiid mite predator (*Galendromus occidentalis* [Nesbitt]) • Life stage: adult and larvae • Source: *G. occidentalis* colony was started using mites collected from a commercial apple orchard near Bridgeport, Washington, USA.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Replicates: 25 replicate discs of a single female for female bioassay; 20 adult females for oviposit for larval bioassay with each treatment replicated 5 times with 90 to 115 (mean = 102) larvae per concentration. Treatments: Three concentrations of each pesticide, tested in a separate bioassay with a distilled water check.

	Data from the female bioassays were analysed using a logistic regression model using a logit link. Mortality, prey consumption and percentage egg hatch were treated as binomial (live/dead, eaten/uneaten, hatched/unhatched) with the binomial distribution specified in the model statement. Data were corrected for zeros by adding 0.125 to the frequency of all outcomes. Eggs and live larvae produced per female were analysed with the same procedure, except that the Poisson distribution (non-negative count data) was specified and 0.25 was added to the all observations to correct for zeros. Concentrations within pesticides were compared when the overall x^2 was significant using pairwise single degree-of-freedom likelihood ratio contrasts ($P > 0.05$). Additional calculations were done to characterise each pesticide on a uniform scale (0-100 or -100) relative to its own control. Mortality was corrected for the control mortality using Abbott's formula (Abbott, 1925). An analogous method was used for other variables to calculate percentage reduction from the control [((E - C)/C) *100, where E is the response in the 1x concentration and C is the response of the control]. A rating scheme of low (<25%), moderate (>25 and <75%) and high (>75%) was used to group corrected percentage mortality and percentage reduction from the check.
Biological effects Determined effect concentration, dose response observed	**Female bioassays** Acetamiprid mortality was moderate and all treatments caused higher rates of mortality than the control but were not different from each other. The 2x dose caused a 10-fold reduction in the number of total prey consumer with a 5.6-fold reduction in the 0.1x dose. However, this bioassay does not distinguish between reduced prey consumption due to contaminated prey and a direct effect on females by spray contact. The females, though alive, were lethargic and nonresponsive. The reduction in fecundity was similar in magnitude to the reduction in prey consumption at the two higher doses. No live larvae were produced in the 2x dose and numbers were significantly reduced in the 1x and 0.1 x doses although all eggs hatched. **Table 1: Mortality, prey consumption, fecundity, egg hatch and larval survival of *G. occidentalis* treated topically as an adult female for acetamiprid**

mg a.s./L	n	% mortality	n	consumed	n	eggs laid	n	% egg hatch	n	live larvae
357	25	36 ± 9.8	25	0.07 ± 0.01	25	0.44 ± 0.13	9	100 ± 0	25	0 ± 0
179	25	32 ± 9.52	24	0.09 ± 0.01	25	0.6 ± 0.12	14	100 ± 0	25	0.04 ± 0.04
18	25	40 ± 10	25	0.11 ± 0.01	25	1.28 ± 0.14	22	100 ± 0	25	0.4 ± 0.15

	<table><tr><td>0</td><td>25</td><td>0 ± 0</td><td>22</td><td>0.75 ± 0.03</td><td>25</td><td>3.88 ± 0.32</td><td>24</td><td>100 ± 0</td><td>25</td><td>3.68 ± 0.34</td></tr></table> **Larval bioassays** Acetamiprid was moderately toxic with one or more doses significantly higher than the control. **Table 2: Mean percentage mortality of *G. occidentalis* larvae following contact/residual exposure to acetamiprid** <table><tr><th>Pesticide</th><th colspan="4">% mortality (± SEM)</th></tr><tr><th></th><th>2x</th><th>1x</th><th>0.1x</th><th>Control</th></tr><tr><td>Acetamiprid</td><td>45.56 ± 2.72</td><td>37.78 ± 6.43</td><td>6.67 ± 2.72</td><td>3.33 ± 1.36</td></tr></table>
Overall assessment	In female bioassays, acetamiprid mortality was moderate and all treatments caused higher rates of mortality than the control. In larval bioassays, acetamiprid was moderately toxic. The study is considered of limited reliability as no analytical verification of the test item was performed. In addition, the study was not performed to a recognised guideline.

Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates	
KCA 8.2.4; KCA 8.2.5	
Author(s)	Beketov, M.A., Liess, M.
Year	2008
Journal	Arch Environ Contam Toxicol Vol. 55, pp. 247–253
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Acute toxicity and drift-initiating action of eleven pesticides was investigated against amphipods (*Gammarus pulex*), blackfly larvae (*Simulium latigonium*) and mayfly larvae (*Baetis rhodani*). Six out of 11 pesticides, including acetamiprid, can initiate drift of macroinvertebrates at sublethal concentrations 7-22 times lower than acute LC_{50} values. Drift of tested animals was detected within 2 h of contamination.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	OECD (1997) guidelines (OECD Guidelines for Testing of Chemicals: Daphnia magna Reproduction Test, vol. 211. Paris, France, pp. 1 – 21)
Test substance Identification of test substance, source, purity, stability	Analytical-grade powder of acetamiprid.. Stock solutions were made in dimethyl sulphoxide (DMSO) with maximum concentration of <1% of DMSO in the exposure solutions.
Test conditions Temperature, pH,	• Temperature: 15 ± 2°C • Photoperiod: 10:14 h (light:dark)

oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Number of animals: 10 per treatment (acute toxicity test) placed in 100 mL glass beakers with 60 mL of test solution • Number of animals: 10 per treatment (microcosm test) placed in 4 glass channels 1.2 m long, 10.5 cm high and 4.5 cm wide; current velocity 0.06 m/s (± 0.001) and discharge was 0.07 L/s (± 0.001); designed as closed circulation system with 5 L of water. • Food availability: None during acute toxicity test • M7 test medium: pH 7.4, conductivity 600 µS/cm, carbonate hardness ~180 mg $CaCO_3$/L.
Controls Positive control, negative control	None stated
Dosing system Exposure (dose, duration, frequency)	• Acetamiprid dose: 0.5 µg/L for *B. rhodani*, 0.5 µg/L for *S. latigonium*, 3 µg/L for *G. pulex* in acute tests (not measured due to technical reasons) • Concentrations used in the microcosm drift experiments were approximately 10x lower than the LC_{50} values from the acute tests • Duration: 96 h (acute toxicity test) • Observations at 0.5, 1, 2, 4, 22, 24, 26, 28 and 48 h after contamination for microcosm test • Exposure solution for acute test made using M7 medium (OECD, 1997)
Test species Body weight or length, gender, age/life stage, source	• Amphipod *G. pulex* and mayfly larvae *B. rhodani* were collected in a small uncontaminated stream near Pulsnitz city (Saxony, Germany) • Larvae of blackfly *S. latigonium* were obtained from uncontaminated stream mesocosm of the Helmholtz Centre for Environmental Research • After collection, the animals were transferred to the laboratory and kept there for acclimation in a 1:1 mixture of M7 medium (OECD, 1997) and water from the stream or mesocosms from which the animals were collected • Life stage: larvae
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Ten organisms per treatment including control Replicates: Not specified for acute test Median LC_{50}s were calculated by Trimmed Spearman–Karber method (Hamilton et al., 1977) using the program Spearman. In microcosm experiments, drift was assessed as the proportion of individuals in the most downstream section compared with that in all other upstream sections considered as a single section. The significance of differences (p <0.05) from the respective controls was assessed as the proportion of drifted/not drifted individuals for each observational time point by contingency tables with the chi-square test. Statistical analyses were performed using Prism 4.0c for Macintosh.

Biological effects
Determined effect concentration, dose response observed

Table 1: Median LC50 values

	96 h LC50 (µg/L)		
	B. rhodani	*S. latigonium*	*G. pulex*
Acetamiprid	NA	3.73	50
Iprodione	NA	480	3460

*LC_{50} for 48 h (>10% mortality in control after 48 h)
NA – not assessed because number of animals was limited

The insect species *B. rhodani* and *S. latigonium* were more sensitive than the amphipod G. pulex to all the tested pesticides except fenvalerate, for which sensitivity of the insects and crustaceans were equal

In microcosm test: six out of 11 tested pesticides initiated drift of stream-dwelling macroinvertebrates at concentrations that caused no significant mortality of the test animals including acetamiprid. Drift of test animals was detected within 2 h of contamination. Maximum drift percentages were detected 4 h after contamination. Drift responses were less pronounced in subsequent observation periods (22-48 h after contamination).

Maximum observed percentage of drifted animals from acetamiprid exposure in microcosm test was approximately (interpreted from graphs) 58% for *B. rhodani*, 30% for *S. latigonium* and 19% for *G. pulex* with only *B. rhodani* being significantly different from controls. The range of concentrations at which drift was observed in the experiments was 7–22 times lower than the respective acute LC_{50} values for all pesticides.

Overall assessment

Six out of 11 pesticides, including acetamiprid, can initiate drift of macroinvertebrates at sublethal concentrations 7-22 times lower than acute LC_{50} values. Drift of tested animals was detected within 2 h of contamination.

The study is considered of limited reliability as no analytical verification of the test item was performed and concentrations where drift was observed in microcosm test not specified.

Comparative toxicities and synergism of apple orchard pesticides to *Apis mellifera* (L.) and *Osmia cornifrons* (Radoszkowski)	
II 8.3.1.1	
Author(s)	Biddinger, D.J., Robertson, J.L., Mullin, C., Frazier, J., Ashcraft, S.A., Rajotte, E.G., Joshi, N.K., Vaughn, M.
Year	2013
Journal	PLoS ONE Vol. 8(9): e72587. doi:10.1371/journal.pone.0072587
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no	Not applicable

reliability	
Summary	Topical toxicities of five commercial grade pesticides, including acetamiprid (Assail 30SG), commonly sprayed in apple orchards were estimated on adult worker honey bees, *Apis mellifera* (L.) and Japanese orchard bees, *Osmia cornifrons* (Radoszkowski). At least 5 doses of each chemical were applied to freshly-enclosed adult bees. Mortality was assessed after 48 hr. Dose-mortality regressions were analysed by probit analysis to test the hypotheses of parallelism and equality by likelihood ratio tests. For *A. mellifera*, the decreasing order of toxicity at LD_{50} was imidacloprid, lambda-cyhalothrin, dimethoate, phosmet and acetamiprid. For *O. cornifrons*, the decreasing order of toxicity at LD_{50} was dimethoate, lambda-cyhalothrin, imidacloprid, acetamiprid and phosmet. Interaction of imidacloprid or acetamiprid with the fungicide fenbuconazole was also tested in a 1:1 proportion for each species. Estimates of response parameters for each mixture component applied to each species were compared with dose-response data for each mixture in statistical tests of the hypothesis of independent joint action. For each mixture, the interaction of fenbuconazole (a material non-toxic to both species) was significant and positive along the entire line for the pesticide. Our results clearly show that responses of *A. mellifera* cannot be extrapolated to responses of *O. cornifrons* and that synergism of neonicotinoid insecticides and fungicides occurs using formulated product in mixtures as they are commonly applied in apple orchards
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Assail 30SG (acetamiprid 30%), Dimethoate 4EC (dimethoate 43.5%), Imidan 70W (phosmet 70%), Provado 1.6F (imidacloprid 17.4%) and Warrior II (lambda- cyhalothrin 22.8%). Interactions of the fungicide Indar 2F (fenbuconazole 22.86%) were also tested.
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Cocoons containing the overwintering *O. cornifrons* adults were removed and refrigerated at 3ºC until 1 April to ensure that their chilling requirements had been met. Loose cocoons were then held inside an incubator (25ºC, constant darkness) until adults emerged. Adults were held in darkness until treated 24–72 h after emergence (24 h for males; 24–72 h for females). Treatment cages were made of a Petri dish (100x20 mm) encasing a 100 mm-long wire mesh cylinder constructed of hardware cloth (with 3x3 mm openings). Glass vial for *ad libitum* feeding of a 50% sucrose solution was included. Six cages were placed on their sides inside a plastic container, which also contained a moist paper towel and a jar of a saturated NaCl solution. Relative humidity was ~75%

Controls Positive control, negative control	Control: water only
Dosing system Exposure (dose, duration, frequency)	Six doses of each pesticide plus a control (water only) were tested in each replication per pesticide per bee species. 1 µL/bee was applied with a Hamilton repeating dispenser that held a 50 µL syringe.
Test species Body weight or length, gender, age/life stage, source	*O. cornifrons* were purchased from a single source in Wisconsin where they had been reared in an organic apple orchard. Larvae were reared in natural *Phragmites* reed bundles and in wooden blocks lined with paper straws. *A. mellifera* used in were purchased as new packages from Gardner Apiaries, Spell Bee. Packages were put into hives pre-sterilised by irradiation. Colonies were established in the spring and kept in an isolated area at least 6 km from any pesticide applications.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Each replication included a total of 60–135 bees of each species depending on species' availability. Dose-mortality regressions were estimated assuming the normal distribution (i.e., probit model) with the computer program PoloPlus as described by Robertson et al. (2007). In a two-step procedure for data analysis, the first step involved plots of standardised residuals being examined for outliers which were then eliminated. The second step involved probit analysis to test hypotheses of parallelism and equality with likelihood ratio tests. PoloPlus also calculated Lethal Dose Ratios (LDRs) which are a means to determine whether two lethal doses are significantly different.
Biological effects Determined effect concentration, dose response observed	The decreasing order of toxicity of the pesticides tested to *O. cornifrons* is dimethoate>lambda-cyhalothrin>imidacloprid> acetamiprid>phosmet. The decreasing order of toxicity of the pesticides tested to *A. mellifera* is imidacloprid>lambda-cyhalothrin = dimethoate> phosmet>acetamiprid. LD_{50} values for acetamiprid were 64.6 (95% CL 38.1 – 252) µg/bee for *A. mellifera* and 4.0 (95% CL 1.1 – 7.1) µg/bee for *O. cornifrons*.
Overall assessment	The study investigated the toxicity of insecticides to *A. mellifera* and *O. cornifrons* but is considered of limited reliability as no analytical verification of the test item was performed and it was not performed to a recognised guideline.

Effects of ten pesticides to *Anystis baccarum* (Acari: Anystidae)	
KCA 8.3.2	
Author(s)	Bostanian, N.J., Laurin, M-C.
Year	2008
Journal	IOBC/WPRS Bulletin Vol. 35, pp. 96-100
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no	Not applicable

reliability	
Summary	This study evaluated the residual toxicity of acetamiprid and other insecticides to *Anystis baccarum*, a common predatory mite in apple orchards and in vineyards of Quebec, Canada. Plastic petri dishes were used for the treatment of the mites and mortality was assessed at 48 h. Acetamiprid caused no residual toxicity to adult *A. baccarum* at a field rate of 0.1543 g a.i./L following 48 h of exposure. A laboratory evaluation in 48 h Petri dish bioassays showed acetamiprid was non-toxic.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Acetamiprid (Assail® 70WP
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 21°C • Relative humidity: 80% • Photoperiod: 16:8 light:dark Each petri dish contained one mite due to their cannibalistic behaviour.
Controls Positive control, negative control	None specified
Dosing system Exposure (dose, duration, frequency)	Plastic petri dishes (50 mm in diameter) were used as cages for the treatment of the mites with a thin-layer chromatography sprayer set at 10.3 kPa used to apply the pesticides. The concentration for acetamiprid was based on dilute application of 1000 L/ha. Mites were released into the treated petri dishes after drying of the residues. Mortality counts were made at 48 h.
Test species Body weight or length, gender, age/life stage, source	*Anystis baccarum* adults were collected from the AAFC experimental farm at Frelighsburg, Quebec. Acaricides and insecticides were not applied in this block for two seasons prior to this study. Field collection was made by tapping branches with *A. baccarum* into a 5 litre bucket and then transferring individual mites into a 30 ml plastic cup
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Two replicates with 30 mites per replicate were used and no statistical analysis was performed on non-toxic pesticides including acetamiprid
Biological effects	Even when applied at several fold the recommended field rate,

Determined effect concentration, dose response observed	acetamiprid caused no residual toxicity to adult *A. baccarum.*
Overall assessment	The study is considered of limited reliability as no analytical verification of the test item was performed and controls were not specified. In addition, the study was not performed to a recognised guideline.

Effect of seven new orchard pesticides on *Galendromus occidentalis* in laboratory studies	
KCA 8.3.2	
Author(s)	Bostonian, N.J., Thistlewood, H.A., Hardman, J.M., Laurin, M., Racette, G.
Year	2009
Journal	Pest Manag Sci Vol. 65, pp. 635–639
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	This study evaluated the toxicity of acetamiprid (and other pesticides) to the predatory mite, *Galendromus occidentalis*. The pesticides were applied to *G. occidentalis*, its prey (*T. urticae*) and the interior substrate (leaf disc and wet cotton strand) in petri dishes in a "worst case laboratory exposure." Compounds were evaluated at their recommended label concentrations and LC_{50} values were estimated. Survival of adults and the number of eggs laid were recorded at 24, 48 and 72 h post treatment. Repellence (mean number of escapees) was also evaluated at 24, 48 and 72 h. Toxicity to freshly laid eggs was evaluated after 144 h. Acetamiprid was not toxic to freshly laid eggs but highly toxic to adults and significantly reduced fecundity. Mortality ranged from 63.7% at 1x to 83.1% at 4x exposures and increased with increasing dose. The LC_{50} for acetamiprid was 0.021 g a.i./L. At 72 h, the average number of eggs laid per female per day was 0.0 and repellence was evaluated at 50% cumulative escapees at the 0.1167 g a.i./L exposure.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Acetamiprid700 g/kg WP (Assail® 70 WP; Dupont Canada)
Test conditions Temperature, pH, oxygen concentration, water hardness,	• Temperature: 24ºC • Relative humidity: 70% • Photoperiod: 16 h:8h, light:dark

conductivity, photoperiod, light intensity, number of animals, food availability	Leaf discs were provided with more than sufficient *T. urticae* of all stages for feeding of *G. occidentalis* to satiation, prior to testing.
Controls Positive control, negative control	Not specified
Dosing system Exposure (dose, duration, frequency)	Pesticides were applied with a thin-layer chromatography sprayer set at 10.34 kPa to *G. occidentalis*, its prey (*Tetranychus urticae*) and the interior substrate (leaf disc and wet cotton strand) in 14.5 cm diameter petri dishes. The test materials were evaluated at their recommended label concentrations on the premise of an application of 600 L sprayable material/ha. For adult mortality, an adult 24 h old female *G. occidentalis* was placed on the lower side of a bean leaf disc (20 mm) infested with *T. urticae* that had been placed with its upper side on a thin wet bed of cotton in a petri dish. Each petri dish contained 13 discs and 52 discs comprised a replicate. The entire set-up was treated (worst-case exposure) with the pesticide at label concentration and replicated 3 times for a total of 156 mites. Survival of adults and the number of eggs laid were recorded at 24, 48 and 72 h post treatment. Other concentrations as multiples above and below the label concentration were evaluated to estimate the LC_{50} values. Escapees (repellence) were evaluated by counting mites at 24, 48 and 72 h following treatment of leaf discs. For treatment of eggs, 2 to 3 adult female *G. occidentalis* were released on the lower side of a bean leaf disc (20 mm) infested with *T. urticae* that had been placed with its upper side on a thin wet bed of cotton in a petri dish (14.5 cm diameter); predators were removed 48 h later and the number of eggs on each leaf was recorded. Each replicate consisted of seven discs infested with spider mites and the eggs of the predator. The experiment was replicated 3 times, with a mean of 32.2 ± 1.4 eggs per replicate.
Test species Body weight or length, gender, age/life stage, source	*Galendromus occidentalis* were collected from IPM orchards in the Okanagan Valley, British Columbia and subsequently reared on two-spotted spider mites, *Tetranychus urticae* Koch, on bush bean leaves in a growth chamber, as described for *N. fallacis* by Rock and Yeargan (1970)
Statistical analyses Sample size/replicates, statistical analysis of data (significance level,	Egg and adult mortality data were corrected according to Henderson and Tilton (1955). LC_{50} values were estimated with probit analyses using Polo PC. Mortality rates for eggs and adults and repellence were arcsine transformed before analysis of variance. Fecundity data

variability)	were log transformed before ANOVA. ANOVA and the Tukey-Kramer test for means separation were carried out with the JMP Statistics and Graphics Guide
Biological effects Determined effect concentration, dose response observed	None of the pesticides tested were toxic to freshly laid eggs after 144 h following treatment. Corrected cumulative percentage mortalities was 2.0 for acetamiprid. Acetamiprid can be classified as highly toxic to *G. occidentalis* with a field concentration of 5.6-fold the estimated LC50 value of 0.021 g a.i./L. For acetamiprid, the average number of eggs laid per female per day was 0.0 at 72 h and repellence was evaluated at 50% cumulative escapees at the 0.1167 g a.i./L exposure. At 48 h after treatment, acetamiprid--treated mites laid the fewest eggs. At 72 h post-treatment acetamiprid still had the greatest negative impact on egg production and there were significantly more escapees than the control, recorded in repellence of the pesticides to adult females, expressed as the mean numbers of escapees (females caught in wet cotton surrounding the disc).
Overall assessment	The study is considered of limited reliability as no analytical verification of the test item was performed and controls were not specified and it was not performed to a recognised guideline.

Effects of six selected orchard insecticides on *Neoseiulus fallacis* (Acari: Phytoseiidae) in the laboratory	
KCA 8.3.2	
Author(s)	Bostanian, N.J., Hardman, J.M., Thistlewood, H.A., Racette, G.
Year	2010
Journal	Pest Manag Sci Vol. 66, pp. 1263–1267
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	This study evaluated the effects of six insecticides including acetamiprid on the survival and egg mortality of *Neoseiulus fallacis* (Garman). The overall egg mortality caused by the six insecticides was negligible as it extended from 0 to 12.1%. Acetamiprid was classified as marginally toxic and its label rates was 0.99-fold the LC50 for adults. Acetamiprid was mildly toxic to at least one growth stage of *N. fallacis*.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Methods of Bostanian et al. (2009) to simulate a "worst-case laboratory exposure"
Test substance Identification of test	**Table 1: Test materials** **Chemical name** | **Product name** | **Active concentration**

substance, source, purity, stability			**(g a.i./L)**
	Acetamiprid	Assail® 70 WP	0.154
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Apple leaf discs were pre-infested and provided with more than sufficient *T. urticae* of all stages for feeding of *N. fallacis* to satiation, prior to testing. Treated predators were held in a growth chamber set at 21°C, 82% relative humidity and 16:8 h light:dark photoperiod		
Controls Positive control, negative control	Control: tap water		
Dosing system Exposure (dose, duration, frequency)	Treatments were made with a thin-layer chromatography sprayer set at 10.34 kPa; the amount of residue of the insecticide suspension throughout the disc was 0.002 mL/cm^2. The test materials were applied to *N. fallacis*, its prey (*T. urticae*) and the interior substrate (leaf disc, 3.1 cm^2) in 14.5 cm diameter petri dishes. Test materials were evaluated at their recommended concentrations as on Canadian labels. LC_{50} values were evaluated as multiples above and below the label concentration. For treatment of eggs, an apple leaf disc was placed with its adaxial (upper) side on a thin wet bed of cotton in a petri dish). Two or 3 adult female *N. fallacis* were released onto the abaxial (lower) side of apple leaf discs. The predators were removed 24 h later and the number of eggs recorded; the number of live eggs was recorded at 120 h after treatment. For adult mortality and fecundity, an adult 48 h old female *N. fallacis* was placed on the abaxial side of an apple leaf disc. Survival of adults and the number of eggs laid were recorded at 24, 48, 72 and 96 h post treatment.		
Test species Body weight or length, gender, age/life stage, source	*Neoseiulus fallacis* was collected from a research orchard in Frelighsburg, Quebec, Canada and reared in the laboratory on two-spotted spider mites, *Tetranychus urticae* Koch.		
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	For adult mortality and fecundity, each petri dish contained 13 discs and 52 discs comprised a replicate. Each treatment was replicated 3 times for a total of 156 predators per concentration. Egg and adult mortality data were corrected according to Henderson and Tilton (1955). LC_{50} values were estimated with probit analyses using Polo PC. Mortality rates for eggs and adults were transformed to arcsine before analysis of variance (ANOVA) and fecundity data were transformed to logarithms. ANOVA and the Tukey–Kramer test for means separation were carried out with the JMP™ software		

	The following scale was used to measure adult mortality: toxic (75–100%), moderately toxic (50–74%), marginally toxic (25–49%) and non-toxic to slightly toxic (0–24%).
Biological effects Determined effect concentration, dose response observed	Egg mortality across all the test materials extended from 0 to 12.1%; acetamiprid had virtually no effect. For acetamiprid, adult mortality ranged from 48.9% at 1x to 93.3% at 4x exposures and increased with increasing dose. The acetamiprid 96 h LC_{50} for adults was determined to be 0.155 g a.i./L and it was classified as marginally toxic with a recommended label rate almost equal to (0.99-fold) the LC_{50} for adults. For fecundity, acetamiprid reduced egg production for only 24 h.
Overall assessment	The study evaluated the effects of six insecticides on the survival and egg mortality of *N. fallacis* study is considered of limited reliability as no analytical verification of the test item was performed and it was not performed to a recognised guideline..

The response of *Neoseiulus fallacis* (Garman) and *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae) to six reduced risk insecticides in Canada	
KCA 8.3.2	
Author(s)	Bostanian, N.J., Hardman, J.M., Thistlewood, H.A., Racette, G.
Year	2010
Journal	Pesticides and Beneficial Organisms IOBC/wprs Bulletin Vol. 55, pp. 73-77
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Laboratory evaluations showed that the response of *Galendromus occidentalis* (Nesbitt) and *Neoseiulus fallacis* (Garman) to insecticides were similar only when a compound was toxic or totally innocuous to the adults and their availability to lay eggs. Compounds in between these two extremes showed moderate responses e.g. acetamiprid was moderately toxic to *G. occidentalis* adults but only marginally toxic to *N. fallacis* adults. It inhibited fecundity considerably in *G. occidentalis* but only had a slight effect on *N. fallacis* fecundity.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Methods of Bostanian et al. (2009) to simulate a “worst-case laboratory exposure”
Test substance Identification of test substance, source,	Acetamiprid (700 g/kg)

<table>
<tr><td>purity, stability</td><td></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>Test conditions not specified</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Control not specified</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>All treatments were made with a thin-layer chromatography sprayer set at 10.34 kPa using the recommended concentration printed on Canadian labels.

A 48 h old adult female predatory mite was placed on the abaxial side of an apple leaf disc (20mm)

Depending on the response of the predators to the label concentration, other concentrations as multiples above and below the label concentration were evaluated to permit an estimation of the LC_{50} values</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Neoseiulus fallacis and Galendromus occidentalis</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Each treatment contained 52 discs replicated three times for a total of 156 predators per concentration.

Adult mortality data were corrected according to Henderson and Tilton (1955). LC_{50} values were estimated by probit analysis using Polo PC. The quotient of the field rate to the LC_{50} value provided additional insight of the toxicity of the compound. To stabilize the variances, mortality rates for adults were transformed to arcsine before analysis of variance (ANOVA) (Clerg et al., 1961). ANOVA and the Tukey- Kramer test for means separation were carried out with JMP™ software.</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Acetamiprid has varied effects on both species.

Table 1: Average % adult female mortality in the laboratory
<table>
<tr><td></td><td colspan="2">G. occidentalis</td><td colspan="2">N. fallacis</td></tr>
<tr><td></td><td>g a.i./L</td><td>% mortality</td><td>g a.i./L</td><td>% mortality</td></tr>
<tr><td>Acetamiprid</td><td>0.117</td><td>63.7</td><td>0.154</td><td>48.9</td></tr>
</table>
Table 2: LC50 values</td></tr>
</table>

	<table><tr><td></td><td colspan="2">*G. occidentalis*</td><td colspan="2">*N. fallacis*</td></tr><tr><td></td><td>LC_{50}</td><td>Field conc./ LC_{50}</td><td>LC_{50}</td><td>Field conc./ LC_{50}</td></tr><tr><td>Acetamiprid</td><td>0.021</td><td>5.6</td><td>0.155</td><td>1.0</td></tr></table> **Table 3: Effects on fecundity at 72 h post-treatment** <table><tr><td></td><td colspan="2">*G. occidentalis*</td><td colspan="2">*N. fallacis*</td></tr><tr><td></td><td>g a.i./L</td><td>%</td><td>g a.i./L</td><td>%</td></tr><tr><td>Acetamiprid</td><td>0.117</td><td>100</td><td>0.154</td><td>23</td></tr></table>
Overall assessment	The study investigated the toxicity of six insecticides to *G. occidentalis N. fallacis*. It is considered of limited reliability as no analytical verification of the test item was performed, test conditions and controls were not specified and information on the source and age of the test species was not provided. In addition, the study was not performed to a recognised guideline.

Toxicity of certain pesticides to the predatory mite *Euseius finlandicus* (Acari: Phytoseiidae)	
KCA 8.3.2	
Author(s)	Broufas, G.D., Pappas, M.L., Vassiliou, G., Koveos, D.S.
Year	2008
Journal	Pesticides and Beneficial Organisms IOBC/wprs Bulletin Vol. 35, pp. 85-91
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The acute and residual toxicity of certain widely used pesticides in plum orchards in Greece to the predatory mite *Euseius finlandicus* were determined with laboratory and semi-field experiments. The acute toxicity of the tested products was evaluated under laboratory conditions using detached bean leaf disks. Subsequently pre-imaginal survival, adult survival and fecundity were determined according to IOBC protocols. Based on mortality and fecundity, acetamiprid was considered as harmful to *E. finlandicus*. The residual toxicity of the tested pesticides to *E. finlandicus* was evaluated using 3 year old potted plum trees (cv. Vanilia) which were sprayed till runoff with a hand sprayer and maintained in the field. At regular time intervals of 3, 7, 10, 15, 20 and 25 days after spraying, leaves were detached from the plants and protonymphs of *E. finlandicus* were transferred on them. Acetamiprid was highly toxic to the predator for more than two weeks, whereas diazinon for 7 to 10 days.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Toxicological tests were carried out using a modification of the detached leaf method (Oomen 1988)

Test substance Identification of test substance, source, purity, stability	Acetamiprid (Profil SG 20), 5 g a.i./hL
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 25ºC • Photoperiod: 16:8 h light:dark • Food availability: *Typha* sp. pollen added to the leaf surface as food for the mites
Controls Positive control, negative control	Control not specified for laboratory bioassays. In extended laboratory bioassays, control trees were sprayed with deionized water.
Dosing system Exposure (dose, duration, frequency)	In the laboratory bioassays, each test unit consisted of a detached bean leaf disc (4cm in diameter) placed upside down on wet cotton wool inside a plastic Petri dish (5cm in diameter). Leaf disks were sprayed with a calibrated Potter Precision Tower producing a wet deposit of 2 mg/cm^2. Residues were allowed to dry for 1 h. In the extended laboratory bioassays, 3 year old potted plum trees were sprayed till run off with a hand sprayer and subsequently maintained in the field. The concentration of the spray solution was adjusted to the maximum recommended rate for field application. At 3, 7, 10, 15, 20 and 25 d following spray application, leaves were cut from the trees and transferred to the laboratory and placed upside down in contact with wet cotton wool inside plastic Petri dishes (5cm in diameter). Each leaf was an experimental unit with 15 protonymphs and 10 replicates, each with 15 protonymphs, were used.
Test species Body weight or length, gender, age/life stage, source	A laboratory stock colony of *E. finlandicus* was established with approximately 350 mites collected from a commercial plum orchard from the area of Alexandria, Northern Greece. Protonymphs were used in the laboratory bioassays.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	On each leaf disc 15 protonymphs were transferred on the leaf surface of the experimental units; for each treatment 10 replicates of 15 predator protonymphs each were used. Cumulative mortality was assessed after exposure for 7 d and percentages were calculated by adding the number of predatory mites which had escaped from the leaf bean disks to those which were dead and compared with the number of mites transferred on the leaf disks at the start of the experiments. Mortality percentages were adjusted for the control mortality using Abbott's formula (Abbott, 1925). Fecundity of the surviving females was assessed from 7th to 14th day following exposure and mean cumulative number of eggs per female

	was calculated as described by Blümel et al. (2002). Additionally, the total effect values (*E*) were calculated, according to Overmeer & Van Zon (1982). Effects were categorised according to the IOBC/WPRS (International Organization for Biological and Integrated Control of Noxious Animal and Plants) classification (Sterk et al., 1999).
Biological effects Determined effect concentration, dose response observed	Acetamiprid was classified as slightly harmful with a slightly persistent toxic effect under field conditions **Table 2: Toxicity to *E. finlandicus*** **Pesticide** | **% mortality** | **% *E*** Acetamiprid | 91.3 | 34
Overall assessment	The study investigated the effect of insecticides on *E. finlandicus* and is considered of limited reliability as no analytical verification of the test item was performed and the control was not specified for the 7 d tests. In addition, the study was not performed to a recognised guideline.

Safety evaluation of eleven insecticides to *Trichogramma nubilale* (Hymenoptera: Trichogrammatidae)	
KCA 8.3.2	
Author(s)	Chen, S., Song, M., Qi, S., Wang, C.
Year	2013
Journal	J. Econ. Entomol. Vol. 106(1), pp. 136-141
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The acute toxicity of acetamiprid and 10 other pesticides to adult *Trichogramma nubilale* was investigated using a dry-film method. The influences of the pesticides on both parasitic ability and different developmental stages were studied using corn leaves residual method, rice moth egg card dipping method and *T. nubilale* parasitized rice moth egg dipping method. Results showed that acetamiprid had different levels of impacts on different developmental stages and could be applied during the pupae stage.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	• Dry-film method (Li et al., 1986) • Corn leaf residue experiments method (Hewa-Kapuge et al., 2003). • Rice moth egg card dipping method (Consoli et al., 2001; Bastos et al., 2006)
Test substance	97% acetamiprid (Hebei Veyong Bio-Chemical Co., Ltd.)

Identification of test substance, source, purity, stability	
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Growth and testing chambers were kept at 24-26^{o}C, relative humidity 72-85%, a photoperiod of 16:8 (L:D) h • CAU 108 corn plants cultivated in greenhouse (three plants/basin, 25-27^{o}C, RH 50-60%) were used for insecticide treatments plants. • In the dry-film tests, *T. nubiliae* were fed with 10% honey water • In the corn leaf residue tests, plants were placed in a greenhouse after treatment at 22-30^{o}C, 50-70% RH and natural lighting
Controls Positive control, negative control	In the dry-film tests, acetone was used as the solvent control. In the corn leaf residue, rice moth egg card and egg, larvae, prepupae and pupae tests, distilled water was the control
Dosing system Exposure (dose, duration, frequency)	In the dry-film tests, 1 mL of each test solution was added into flat-bottomed tube and then the tube was put flatwise and rolled rapidly to make the solution coat the inner wall uniformly. ~100 adult *T. nubilale* (aged 4-6 h after emergence) were added into each tube after the solvent evaporated. The number of the adults and deaths were checked after 24 h and mortality rate was calculated. In the corn leaf residue test, corn was treated with a hand sprayer until liquid dropped down from the leaves. Three pieces of corn leaves ~5 cm in length were cut on the zero, second, fourth and seventh day and each was inserted into a flat-bottom tube filled with 1 cm deep 4% agar; ~30 *T. nubilale* aged 4-6 h after emergence were added into each tube. Number of adults and deaths in each tube was checked at 8 h after treatments and mortality rate was calculated. In the rice moth egg card dip test, egg cards with ~900 fresh rice moth eggs per card were dipped in test solutions for 5 seconds. Cards were dried and then each card was put in a flat-bottomed tube. ~20 *T. nubilale*, aged 4-6 h after emergence, were added to each tube. The number of adults and deaths in each tube was checked, live ones were removed after 24 h and the number of parasitized eggs was checked after 120 h. F_1 generation began to hatch after 188 h, the number of F_1 emergence was checked.
Test species Body weight or length, gender, age/life stage, source	*Trichogramma nubilale* eggs were bought from Guangdong Entomological Institute
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	In the dry-film tests, corn leaf residue, rice moth egg card dipping and the safety evaluation for egg, larvae, prepupae and pupae phase tests, each treatment was repeated 4 times. Statistical analyses: SPSS 12.0 was used to analyse the test data from the dry-film method. LC_{50} and 95% CL was calculated using Probit

	model. Data from other tests were also analysed using SPSS 12.0 to calculate the mean and SE and the significance of difference was tested by Duncan's new multiple range analysis for multiple comparisons.
Biological effects Determined effect concentration, dose response observed	Acetamiprid was the third most toxic pesticide with a LC_{50} of 0.609 mg/L. Mortality of acetamiprid in the corn leaf residue test was 77.5 ± 4.17% at zero days after application of the test material; mortality decreased with increasing time between application of the test material and exposure to the test animals. Effects of acetamiprid on parasitism capacity decreased with increasing time between application of the test material and exposure to the test animals. The average number of parasitic eggs per female of acetamiprid-treated F_0 generation was 16.1, 42.9% lower than control, but survival of F_0 and the emergence of F_1 were not impacted adversely. Using acetamiprid to treat with prepupae and pupae resulted in no significant difference of emergence rate.
Overall assessment	The study investigated the acute toxicity of acetamiprid and 10 other pesticides to adult *Trichogramma nubilale* along with effects on mortality, parasitism and effects on eggs, larvae, prepupae and pupae. The study is considered of limited reliability as no analytical verification of the test item was performed and it was not performed to a recognised guideline.

Impact of selected pesticides used to protect strawberries for predatory mite *Amblyseius andersoni* (Chant) survival	
KCA 8.3.2	
Author(s)	Chorazy, A; Garnis, J.
Year	2011
Journal	Progress in Plant Protection Vol. 51(2), pp. 900-904
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this research were to determine the toxicity of several pesticides to *Amblyseius andersoni* using direct contact and residual toxicity tests. Results with Mopsilan 20 SP did not show any significant difference in survival depending on 24, 72 or 120 hrs time elapsed before organisms were added to treated strawberry leaves.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No specific protocol cited, but detailed methods are reported
Test substance Identification of test substance, source, purity, stability	**Commercial Product**: Mospilan 20 SP **Active Ingredient**: Acetamiprid

Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Temperature: 25 ºC 75% relative humidity 16:8 h light:dark photoperiod 5 organisms per replicate
Controls Positive control, negative control	Negative Control: Water
Dosing system Exposure (dose, duration, frequency)	Direct Toxicity Tests: 5 *A. andersoni* were applied to strawberry leaves of the Honeoye variety and placed in Petri dishes. The leaves were then subjected to a spraying treatment via a fine-droplet sprayer. The experiment was carried out in 20 repetitions and observations of mortality were made 24 and 48 h post treatment. Long-Term Exposure Toxicity Tests: Leaves of the strawberry plant were sprayed with the same products and concentrations previously, but 24, 72 and 120 h post spraying 5 organisms were placed on the treated leaves. The experiments were carried out in 15 repetitions and observations of mortality were made 24, 48, 72 and 96 h after the organisms were added to the leaves. Concentrations: All pesticides were tested at field application rates as recommended by their manufacturers (no other information is reported).
Test species Body weight or length, gender, age/life stage, source	*Amblyseius andersoni*, no other information is reported
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Data were analysed using ANOVA single-factor variance analysis. The detailed comparison of mean values were done using the LSD test at a level of $\alpha = 0.05$.
Biological effects Determined effect concentration, dose response observed	**Table 1: Direct toxicity of pesticides to *A. andersoni*** **Plant Protection Agent** / **Mean Mortality [%] After Time**: **24 hours**, **48 hours** Control: 3 a, 8 a Mospilan 20 SP: 87 b, 91 b Values followed by the same letter in a column do not differ significantly. **Table 2: Mean mortality of *A. andersoni* following chemical protection agent application depending on predator introduction time** **Plant** / **Predator** / **Mean** / **Mean**

	<table><tr><th>Protection Agent</th><th>Introduction Time [days]</th><th>Mortality at Time*</th><th>Mortality Rate** [%]</th></tr><tr><td rowspan="3">Control</td><td>1</td><td>8 a</td><td rowspan="3">9 a</td></tr><tr><td>3</td><td>9 a</td></tr><tr><td>5</td><td>11 a</td></tr><tr><td rowspan="3">Mospilan 20 SP</td><td>1</td><td>89 a</td><td rowspan="3">89 b</td></tr><tr><td>3</td><td>93 a</td></tr><tr><td>5</td><td>84 a</td></tr></table> Values followed by the same letter do not differ significantly * comparison for given product ** product comparison
Overall assessment	Due to three key deficiencies, this article should be considered with 'limited reliability': • There is no analytical data to support the concentrations tested. • A dilution series is not used; the pesticides were only tested at manufacturers recommended application rates. The application rates are not reported in the article. • Not complete reporting of some methods and data analysis

Effect of insecticides on mealybug destroyer (Coleoptera: Coccinellidae) and parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), natural enemies of citrus mealybug (Homoptera: Pseudococcidae)	
KCA 8.3.2	
Author(s)	Cloyd, R.A., Dickinson, A.
Year	2006
Journal	J. Econ. Entomol. Vol. 99(5), pp. 1596-1604
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The direct (lethal or acute effects) and indirect (sublethal) effects of insecticides including acetamiprid on the mealybug destroyer, *Cryptolaemus montrouzieri* Mulsant and the parasitoid *Leptomastix dactylopii* Howard, natural enemies of citrus mealybug, *Planococcus citri* (Risso), were evaluated in the laboratory. The adult stages of both natural enemies were exposed to sprays of insecticides at label-recommended rates to assess direct mortality after 24, 48 and 72 h, respectively. The effects of the insecticides on *L. dactylopii* parasitization rate and percentage of parasitoid emergence also were monitored using the label and 4x the recommended label rate. At 4x the recommended label rate, acetamiprid was harmful to the parasitoid with 100% mortality 72 h after application. Acetamiprid, were toxic to *C. montrouzieri* adults with 100% mortality after 48 h.
Reliability check:	
Parameter	**Information available**

Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Acetamiprid
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	The parasitoids were maintained for the test at a temperature of 20 ± 2°C and a photoperiod of 14:10 (L:D) h. A honey-water solution [50:50 (vol:vol)] was freely available to *L. dactylopii*. Newly emerged (<24-h-old) male and female *L. dactylopii* were collected from the laboratory colony by using an aspirator and allowed to mate for 24 h in 9-dram vials with a drop (0.05 ml) of honey-water solution [50:50 (vol:vol)] applied to the inside portion of the lid.
Controls Positive control, negative control	Untreated check (dried filter paper) and deionized water control
Dosing system Exposure (dose, duration, frequency)	A 100 x 20 mm glass petri dish was inverted and Whatman No. 1 filter paper was placed in the bottom of the petri dish. The petri dish and filter paper were sprayed once with 0.8 ml of each treatment solution by using a 946-ml spray bottle calibrated so that the full spray trajectory thoroughly moistened the entire petri dish and filter paper. A single-mated female parasitoid was placed into each petri dish immediately after the treatments had been applied. Each female parasitoid was monitored after 24, 48 and 72 h to assess whether they were alive or dead. Insecticide rates used were based on the label recommendations from the manufacturer; acetamiprid was applied at 0.18 g/946 ml and also at 4x the recommended label rate, 0.72 g/946 ml. For the indirect effects on parasitization rate and sex ratio, 100 x 20 mm glass petri dishes were inverted and Whatman No. 1 filter paper was placed in the bottom of the petri dish. Citrus mealybugs were added to the petri dishes and then sprayed once with 0.8 ml of each treatment solution by using a 946-ml spray bottle. A single-mated female parasitoid was placed into each petri dish immediately after the treatments had been applied. Petri dishes were checked daily for 2 weeks after mealybugs had been parasitized (mummified), to assess adult parasitoid emergence. Once emergence began, petri dishes were monitored daily and the parasitoids were collected and sexed using antennal morphology. The experiment was terminated 2 d after no more adult parasitoids emerged from the parasitized mealybugs

<table>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>The parasitoid L. dactylopii Howard was obtained from a laboratory colony reared on citrus mealybugs feeding on butternut squash, Cucurbita maxima (L.), maintained in an environmental chamber.

Adult C. montrouzieri were obtained from a commercial supplier of biological control agents (IPM Laboratories, Inc., Locke, NY).</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Randomized design with eight treatments and five replications per treatment for the label rate experiment.

Eight treatments with 10 replications per treatment for 4x the label rate experiment and for the direct effects on C. montrouzieri experiment.

Randomized design with seven treatments and five replications per treatment, per release time (n = 2), for a total of 70 replicates for the indirect effects on parasitization rate and sex ratio at the label rate.

Six treatments with five replications per treatment, per release time (n = 2) for a total of 60 replicates for the indirect effects on parasitization rate and sex ratio at 4x label rate.

For the L. dactylopii direct effects experiments, all data were analysed using a logistic regression repeated measures procedure, which combined both the 24 and 48 h time intervals. A PROC GENMOD program, which is a generalised linear model program, was used to analyse the data in a SAS Statistical Software Program with treatment as the main effect.

For the L. dactylopii indirect effects experiments all data were analysed using a one-way analysis of variance (SAS Institute 2002) with treatment as the main effect. Significant treatment means were separated using a Fisher’s protected least significant difference (LSD) test at $P = 0.05$.

For the C. montrouzieri experiments, all data were analysed using a logistic regression repeated measures procedure. A PROC GENMOD program, which is a generalized linear model program, was used to analyse the data in a SAS Statistical Software Program.</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Percentage of mortality of L. dactylopii at recommended rate
<table>
<tr><td></td><td colspan="3">% mortality</td></tr>
<tr><td>Treatment</td><td>24 h</td><td>48 h</td><td>72 h</td></tr>
<tr><td>Acetamiprid</td><td>20</td><td>20</td><td>20</td></tr>
</table>
Table 2: Percentage of mortality of L. dactylopii at 4 x recommended rate
<table>
<tr><td></td><td colspan="3">% mortality</td></tr>
<tr><td>Treatment</td><td>24 h</td><td>48 h</td><td>72 h</td></tr>
</table></td></tr>
</table>

	<table><tr><td>Acetamiprid</td><td>100</td><td>100</td><td>100</td></tr></table> For the direct effects on *L. dactylopii* at the label rate, acetamiprid at 0.18 g/946 ml resulted in 20% mortality after 24, 48 and 72 h. For the direct effects on *L. dactylopii* at 4x the label rate, acetamiprid at 0.72 g/946 ml resulted in 100% mortality after 24, 48 and 72 h. At both the recommended and 4x recommended label rates, treatment was significant for parasitisation rate and percentage of parasitoid emergence but not sex ratio of *L. dactylopii* at both immediate and 24 h timescales. **Table 3: Percentage of mortality of *C. montrouzieri* at recommended rate** <table><tr><td></td><td colspan="2">% mortality</td></tr><tr><td>Treatment</td><td>24 h</td><td>48 h</td></tr><tr><td>Acetamiprid</td><td>70</td><td>100</td></tr></table> Acetamiprid was highly toxic to adult *C. montrouzieri* causing 70% mortality at 0.18 g/946 ml after 24 h and 100% mortality after 48 h.
Overall assessment	The study is considered of limited reliability as no analytical verification of the test item was performed and the study was not performed to a standardised guideline.

Toxicity of insecticides used in the Brazilian melon crop to the honey bee *Apis mellifera* under laboratory conditions	
KCA 8.3.1	
Author(s)	Costa, E.M., Araujo, E.L., Maia, A.V.P., Silva, F.E.L., Bezerra, C.E.S., Silva, J.G.
Year	2014
Journal	Apidologie Vol. 45, pp. 34–44
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The toxicity of insecticides, including acetamiprid, used in melon crop (*Cucumis melo* L.) on adults of *Apis mellifera* L. was evaluated under laboratory conditions. Three experiments were conducted: 1) direct spraying, 2) insecticide contaminated diet and 3) contact with insecticide contaminated melon leaves. Bees were exposed to nine insecticides at the highest dosages recommended by the manufacturers for the melon crop in Brazil. Acetamiprid was most toxic when directly sprayed on the bees.
Reliability check:	
Parameter	**Information available**

Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Acetamiprid.
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Climate-controlled growth chamber at 25 ± 2°C, 50 ± 10 % RH and photophase of 12 h. Bees were offered a solution of honey and sugar in a plastic vial and also a cotton wick saturated with distilled water
Controls Positive control, negative control	As control treatments, in the first assay (direct spraying), water was directly sprayed on the bees; in the second assay (insecticide contaminated diet), the control treatment comprised only the solution of honey and sugar and in the third assay (contact with insecticide contaminated melon leaves), water was sprayed over the leaves.
Dosing system Exposure (dose, duration, frequency)	Three ways of exposure were used to evaluate the toxicity of insecticides on bees: directly spraying on adult bees, feeding bees with insecticide contaminated diet and bee contact with insecticide residues on treated melon leaves. Bees were placed in plastic containers (cylinders of 12.0 cm diameter × 9.0 cm height) (hereon called arenas) covered with voile cloth secured with rubber bands. Each arena constituted an experimental unit. The acetamiprid dose was 0.060 g a.i./L, maximum recommended dosage, prepared in distilled water. Mortality was assessed at 1, 2, 3, 4, 5, 6, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60 and 72 h after insecticide treatment and the behaviour (e.g., prostration, tremors, paralysis, etc.) of the bees was monitored and recorded from the first 30 min after spraying until the end of the experiment. In the first assay (direct spraying), groups of 10 bees were directly sprayed with the test material using a manual sprayer at 0.58 mL/s and an average spraying rate of 0.00583 mL/cm^2, simulating a field spraying. Bees were then placed in the arenas to assess the effects of insecticides until the end of the 72 h period. In the second assay (insecticide contaminated diet), diet (bee candy) was prepared using 20 mL of honey and 50 g of sugar, which were

<table>
<tr><td></td><td>mixed and homogenized to form a paste. The insecticides were applied to the diet surface (7.06 cm^2) to simulate a field spraying.

In the third assay (contact with insecticide contaminated leaves), melon plants of the variety Orange Flesh were cultivated in a greenhouse. Plants with a minimum of four true leaves were selected and five plants were used for each treatment. Using a manual sprayer at 0.58 mL/s and an average spraying rate of 0.00583 mL/cm^2, a field spraying was simulated so that the insecticide drops uniformly covered the entire foliar surface. The plants were then transferred to an airy and shaded room for 1 h to allow for the insecticides to dry. Three dry leaves were placed in each arena with the regular diet and water before the insects were released.</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>The adult bees used in the experiments were collected from a single colony at the apiary of the Cooperative APISMEL, in Serra do Mel, in the state of Rio Grande do Norte</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Random design and each way of exposure comprised 10 treatments and 10 replications. Each replicate comprised 10 adult bees.

Statistical analyses: Data for survival of adults were analysed using the package “survival” (Therneau and Lumley, 2010) for the R software and subjected to a Weibull distribution analysis. Treatments with similar effects (toxicity and mortality speed) were grouped using contrasts. The lethal time 50 (LT_{50}) was also calculated for each group. Mortality percentages were calculated for each treatment in the three exposure methods and corrected using Abbott’s equation (Abbott, 1925).</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Mortality of Apis mellifera
<table>
<tr><th></th><th colspan="3">% mortality</th></tr>
<tr><th></th><th>Direct spraying</th><th>Contaminated diet</th><th>Contaminated leaves</th></tr>
<tr><td>Acetamiprid</td><td>100</td><td>47.6</td><td>60</td></tr>
</table>
In the first assay, acetamiprid had a LT_{50} of 6.11 h and caused 95% mortality in the first 15 h of assessment; the corrected (Abbot’s equation) mortality from direct spraying was 100%. In the second assay, 47.6% mortality resulted from exposure via contaminated diet and the LT_{50} was 79.84 h. In the third assay, 60% mortality resulted from exposure to contaminated leaves and the LT_{50} was 60.89 h.</td></tr>
<tr><td>Overall assessment</td><td>The study is considered of limited reliability as no analytical verification of the test item was performed and the study was not performed to a standardised guideline.</td></tr>
</table>

The impact of insecticides applied in apple orchards on the predatory mite *Kampimodromus aberrans* (Acari: Phytoseiidae)
KCA 8.3.2

Author(s)	Duso, C., Ahmad, S., Tirello, P., Pozzebon, A., Klaric, V., Baldessari, M., Malagnini, V., Angeli, G.
Year	2014
Journal	Exp Appl Acarol Vol. 62, pp. 391–414
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Field and laboratory experiments were conducted to evaluate the effects of insecticides, including acetamiprid, on the predatory mite. *Kampimodromus aberrans*. Single or multiple applications of neonicotinoids caused no detrimental effects on predatory mites. In the laboratory, acetamiprid did not affect female survival and was not associated with any effects on fecundity. Acetamiprid caused no negative effects on predatory mites in field trials.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Tirello et al. (2013) for laboratory experiment
Test substance Identification of test substance, source, purity, stability	Acetamiprid
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Laboratory studies: Fresh *Typha* sp. pollen was provided every two days as food for predatory mites. Experimental units were kept in a climatic chamber at 25 ± 2°C, 70 ± 10 % relative humidity and 16L:8D photoperiod
Controls Positive control, negative control	Untreated controls

Dosing system Exposure (dose, duration, frequency)	Field experiments: insecticides applied in apple orchard according to codling moth control timing, sampling was conducted before and approximately every 10 d after insecticide applications. Laboratory experiments: insecticides applied in field trials were tested at the same concentrations in the laboratory assuming a volume of insecticide solution of 10 hL/ha. Apple leaves were treated with insecticides and then mated *K. aberrans* females were transferred onto the leaves to expose them to fresh insecticide residues. Effect of insecticides on female mortality was evaluated after 72 h. Surviving females were observed daily for additional 4 d to assess effects on fecundity. Eggs' hatching was also monitored until 100 % hatching rate was reached in the control.
Test species Body weight or length, gender, age/life stage, source	*Kampimodromus aberrans*
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Field experiment #1: randomized design was adopted with four replicates per treatment (insecticides treated + control). 60 leaves per treatment (15 leaves per replicate) were removed and transferred to the laboratory where predatory and phytophagous mites were counted under a dissecting microscope. Statistical analysis for field experiment #1: Data were analysed with a restricted maximum likelihood (REML) repeated measures model with the SAS MIXED procedure (SAS Institute 1999). Mite densities were considered as response variables with repeated measures made at different times, i.e. sampling dates. Using an F test ($\alpha = 0.05$) the effect of insecticide application, time and their interaction was evaluated. Contrasts ($\alpha = 0.05$) were designed for pairwise comparison between treatments before and after insecticide applications. Degrees of freedom were estimated using the Kenward–Roger method (Littell et al., 1996). According to Akaike's Information Criterion, first-order autoregressive was chosen as best fitting covariance structure for correlating different sampling dates (Littell et al., 1996). Data were checked for analysis assumptions and square-root transformation was applied. Field experiment #2: The number of insecticide applications (1, 2 or 3) was considered as nested effect within each insecticide treatment. The overall experimental design resulted in 20 treatments; completely randomized design with three replicates per treatment was adopted. Statistical analysis field experiment #2: Data were analysed using a REML repeated measures model where treatments, times and their interactions were considered as sources of variation and F tests were used to evaluate their effects ($\alpha = 0.05$). Degrees of freedom were estimated using the Kenward–Roger method (Littell et al., 1996).

	Mite densities were considered as dependent variables with repeated measurements at different times. Data were checked for normality assumption and thus the number of phytoseiids per leaf was log (x + 1) transformed prior to the analyses. The SLICE option of the LSMEANS statement was used to test treatment effect variation during observation periods (SAS Institute, 1999). Differences among treatments were evaluated with a t test with Bonferroni adjustment (α = 0.05) to least square means. Laboratory experiments: 45–50 females tested per insecticide. Statistical analysis involved one-way ANOVA with F test (P = 0.05) to evaluate the effect of insecticides on mite survival, fecundity, escape rate (number of escaped or drowned females/initial number of females) and egg hatching using GLM procedure of SAS (SAS Institute, 1999). Treatments were compared using Tukey–Kramer test (P = 0.05). In order to meet the ANOVA assumptions, data on survival were arcsin-transformed while squareroot transformation was applied to data on fecundity. The Blumel and Hausdorf (2002) formula was used for fecundity calculation. The overall toxicity of each insecticide was expressed as: E = 100% - (100% - M) x R where E is the coefficient of toxicity; M is the corrected mortality according to Abbott (1925); R is the ratio between the average number of hatched eggs produced by treated females and the average number of hatched eggs produced by females in the control group.
Biological effects Determined effect concentration, dose response observed	Acetamiprid did not significantly affect female mite survival, fecundity, escape rate, or egg hatching in the laboratory studies. There were no effects on *K. aberrans* populations from acetamiprid application in the field studies.
Overall assessment	The study evaluated the effects of insecticides on *K. aberrans* but is considered to be of limited reliability as no analytical verification of the test item was performed and the study was not performed to standardised guidelines.

Effects of reduced-risk pesticides and plant growth regulators on rove beetle (Coleoptera: Staphylinidae) adults	
KCA 8.3.2	
Author(s)	Echegaray, E.B., Cloyd, R.A.
Year	2012
Journal	J. Econ. Entomol. Vol. 105(6), pp. 2097-2106
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	This study evaluated the effects of reduced-risk pesticides and plant

	growth regulators on adult rove beetle, *Atheta coriaria* (Kraatz), a biological control agent mainly used against fungus gnats (*Bradysia* spp.). *A. coriaria* survival, development and prey consumption were tested under laboratory conditions. Effects were assessed based on adult survival, as measured by recovery rates of released adults and any additional effects were determined based on impact on rove beetle development time from egg to adult and on prey consumption. Rove beetle survival was consistently higher when adults were released 24 h after, rather than before, applying pesticides. Acetamiprid was harmful to rove beetle adults.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	The effects of pesticides on rove beetle adults were determined following the procedures described by Cloyd et al. (2009).
Test substance Identification of test substance, source, purity, stability	Acetamiprid (TriStar); recommended label rate: 2.66 fl. oz./100 gal, 0.014 g/70 ml; or high rate: 5 fl. oz./100 gal, 0.028 g/70ml
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Laboratory conditions: 22-24ºC; 40-60% relative humidity and a photoperiod of 0:24 (L:D) h. 1.5 g of raw oatmeal was placed on the growing medium surface as a food source for the rove beetle adults.
Controls Positive control, negative control	Deionised water control (70 ml and same food source) was included in all experiments.
Dosing system Exposure (dose, duration, frequency)	Rove beetle adult survival was assessed after exposure to pesticide treatments for 10 d. Effects were determined after rove beetles were applied to the growing medium 24 h before and 24 h after, applying the pesticides. 300 ml of growth medium was placed into a 473 ml deli squat container. Modified lids with insect screening (50 x 24 [0.2 x 0.8 mm]) were used to allow for ventilation. 70 ml of each pesticide solution was applied uniformly as a drench to the growth medium in each deli squat container,
Test species Body weight or length, gender, age/life stage, source	*Atheta coriaria*, adults' rove beetle adults (males and females of various ages) randomly collected from a laboratory-reared colony (maintained at Kansas State University, Manhattan, KS). Rove beetles were originally obtained from an established colony at the University of Illinois and have undergone over 20 generations in culture.
Statistical analyses	Experiments were set-up as a randomized complete block design with

Sample size/replicates, statistical analysis of data (significance level, variability)	two blocks (days as blocks) and 10 replications per treatment. Data from all experiments were analysed using a statistical analysis software program SAS Systems for Windows, version 9.2. Data associated with the effects of pesticides on *A. coriaria* were subjected to an analysis of variance (ANOVA) using the PROC ANOVA procedure with the number of live rove beetle adults as the response variable (main effect). Any significant differences among the treatments were determined using a Tukey's least significant means test at a significance level of $\alpha = 0.05$.
Biological effects Determined effect concentration, dose response observed	**Experiment 1** The number of rove beetle adults recovered from the acetamiprid treatment at the high rate was 10.3 ± 1.3 which was not significantly different from the recovery rate (13.3 ± 0.6) obtained when acetamiprid was applied at the low rate; both were significantly lower than the control. The recovery rate associated with low rate acetamiprid was similar to the soybean and rosemary oil treatment whereas the lowest adult recovery rate was obtained from the high rate acetamiprid treatment
Overall assessment	The study evaluated the effect of pesticides on *A. coriaria* and is considered of limited reliability as no analytical verification of the test item was performed and the study was not performed to a recognised guideline.

Effects of sublethal doses of acetamiprid and thiamethoxam on the behaviour of the honeybee (*Apis mellifera*)	
KCA 8.3.1	
Author(s)	El Hassani, A.K., Dacher, M., Gary, V., Lambin, M., Gauthier, M., Armengaud, C.
Year	2008
Journal	Arch Environ Contam Toxicol Vol. 54, pp. 653–661
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The sublethal effects of acetamiprid and thiamethoxam were studied after acute treatment on the behaviour of the honeybee (*Apis mellifera*) under laboratory conditions. Acetamiprid and thiamethoxam were administered orally and applied topically on the thorax. After oral consumption, acetamiprid increased sensitivity to antennal stimulation by sucrose solutions at the dose of 1 µg/bee and impaired long-term retention of olfactory learning at the dose of 0.1 µg/bee. Acetamiprid thoracic application induced no effect in these behavioural assays, but increased locomotor activity (at 0.1 and 0.5 µg/bee, but not at 1 µg/bee) and water-induced proboscis extension reflex (0.1, 0.5 and 1 µg/bee). Unlike acetamiprid, thiamethoxam had no effect on bees' behaviour under the conditions used. The results suggest a particular vulnerability of honeybee behaviour to sublethal

	doses of acetamiprid.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Locomotor activity was analysed as previously described (Lambin et al. 2001). Classical olfactory conditioning was carried out as previously described by Gerber et al. (1998) and El Hassani et al. (2005).
Test substance Identification of test substance, source, purity, stability	Acetamiprid (99% purity) and thiamethoxam (97% purity). Acetamiprid was dissolved in acetone to obtain the stock solution and thiamethoxam was dissolved in acetonitrile.
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Honeybees were maintained with ad libitum food in small Plexiglas boxes until the beginning of the individual tests and were maintained in an incubator during the night during the experimental period. No other test conditions defined.
Controls Positive control, negative control	For topical treatment, control animals received 1 µl of water containing the solvent (10%). For oral treatment, control animals were fed with 10 µl of sucrose solution containing the solvent (1%).
Dosing system Exposure (dose, duration, frequency)	For topical application, the stock solutions were dissolved in water and 1 µl of the final solution was deposited onto the thorax of the honeybee. For oral treatment, the stock solutions were dissolved in sucrose solution (40%, w/v) that was used to feed honeybees individually with 10 µl. Both test substances were used at doses of 0.1, 0.5, or 1 µg/bee For locomotor experiments, honeybees were directly and individually introduced into a 5 ml syringe where they received oral treatment or topical application of acetamiprid or thiamethoxam and they were left in the syringe until being tested for motor activity. For locomotor activity, honeybees were tested in an open-field-like apparatus (30 x 30 x 4 cm) standing vertically and illuminated from above. The back area was divided into six horizontal levels 5 cm high; each level was divided into squares of 5 x 5 cm. Honeybees were introduced in the bottom right-hand side and were allowed to move for a 3 minute observation period. The position of the animal in a square was recorded every 3 s with a keyboard computer. Variables assessed for each animal were the total distance walked, the duration of immobility and the number of ascents from one level to a higher one. Effects of acetamiprid on locomotor activity were evaluated 60 minutes after a single topical application or oral dose.

	For proboscis extension reflex (PER) to sucrose assays and learning experiments, bees were anesthetized by cooling. They were then fixed in a small tube by depositing a drop of wax-colophony mixture onto the dorsal part of the thorax; the head and the forelegs were left free. The PER was used to sample bees' sensitivity to ascending concentrations of sucrose solution (ACSS) and to examine the dose-dependent relation of orally administered and thoracically applied test material on sucrose responsiveness. Each animal was tested twice with ACSS: 60 min before and 60 min after treatment. The same point of satiety was achieved for orally and topically treated bees by giving animals 10 µl of 40% (w/v) sucrose solution 1 h before each test with ACSS. Allowing bees to drink water ad libitum 1 h before each test with ACSS controlled the effect of thirst on sucrose sensitivity. PER to water was tested 3 min before testing PER to sucrose solution before and after treatment. Concentrations of sucrose solution increased in a $\log_{10}$ series of -1.0, -0.5, 0.0, 0.5, 1.0 and 1.5, corresponding to sucrose concentrations of 0.1%, 0.3%, 1%, 3%, 10% and 30% (w/v). For each concentration, percentage of PER released by honeybees was recorded. Solutions were applied to antennae with a 3 minute intertrial interval. All bees were tested twice with ACSS, but only bees presenting no response to water before the first test with ACSS were included in the statistical analysis of PER to sucrose. For evaluating PER to water, all the honeybees were taken into account. Bees were tested twice: 1 h before and 1 h after treatment, the antennae were touched with a drop of water 3 min before each ACSS. Results for PER to sucrose and PER to water were analysed separately. For olfactory learning tests, a five-trial paradigm with an intertribal interval of 1 min, which leads to long-term memory, was used. Honeybees were trained to associate the conditioned stimulus (CS) represented by a coffee odour with an unconditioned stimulus (US) represented by a drop of sucrose (40% w/v) applied to the antennae. The CS and the US lasted 3 seconds and the US was presented 2 seconds after onset of the CS. No food was allowed to the bee during the training phase until the fifth trial, when a small drop of sucrose (40% w/v) was presented to the proboscis. In the testing trials, the CS was presented alone 1, 24 and 48 h after the learning session and the percentage of bees releasing a conditioned PER was recorded for each delay.
Test species Body weight or length, gender, age/life stage, source	Worker honeybees were caught at the top of outside hives at the Paul Sabatier University Campus, France, or were collected from hives maintained in a warmed apiary.
Statistical analyses Sample size/replicates, statistical analysis of	Experiments were performed in triplicate and repeated at least three times. For locomotor activity, analysis of variance (ANOVA) was conducted to compare the effects of the different doses. PER rates to

data (significance level, variability)	the different sucrose solutions, before and after treatment, were compared for each of the control and treated groups using the McNemar test. For PER rates to water, comparison under different treatments was done using Fisher's exact test. When the *p*-values were significant, pairwise comparisons between all groups were performed. For olfactory learning G tests were used to compare the different doses. All tests were two-tailed and were performed with SPSS12. A difference was considered to be significant when the obtained *p*-value was <0.05.
Biological effects Determined effect concentration, dose response observed	Acetamiprid increased the total length walked in the open-field-like apparatus 1 h after treatment. A significant difference was revealed between controls and 0.1 and 0.5 µg/bee treated bees, topical acetamiprid application including an increase in the distance covered. A significant decrease in the duration of immobility of bees was observed 1 h after topical, but not oral, treatment with 0.1 and 0.5 µg/bee doses. The number of ascents was not affected by acetamiprid. The locomotor activity of bees was not affected by thiamethoxam. A significant difference was seen between orally and topically treated bees with topically treated bees moving less in the box and covering a shorter distance. In honeybees treated orally with 0.1 and 0.5 µg acetamiprid, a significant decrease in sucrose responsiveness compared to the controls was observed. After a thoracic application, the sucrose responsiveness of acetamiprid-treated animals was not modified compared to that of the controls. Bees treated with thiamethoxam presented identical sucrose responsiveness before and after oral or topical treatment whatever the dose. No effect of acetamiprid on water responsiveness was found after oral treatment. Topically applied, acetamiprid induced a dose-dependent increase in the PER to water compared to that of controls. No significant modification of water responsiveness was observed after treatment with thiamethoxam. Orally absorbed acetamiprid induced no significant impairment of animals' performances during learning. However, percentage PER tested 48 h after learning significantly differed across the groups. The performance was significantly lower in the group treated with 0.1 µg/bee than in the control group, whereas the performance of animals which received 0.5 and 1.0 µg did not differ significantly from that of any other group. Topical acetamiprid treatment induced no significant effect on learning and retention performances. The training performance of treated orally with thiamethoxam was not significantly different to that of the controls. A significant increase in performance was observed at the third acquisition trial at 0.5 µg/bee applied topically because of an unusual decrease in PER in the controls in the third trial. No significant effect was observed on

	retrieval performance after thiamethoxam either absorbed orally or applied topically. The doses of pesticides used were in the LD_{50}/100 to LD_{50}/10 range and induced no extra mortality compared to controls. Twenty-four and 48 h after oral or topical contamination, the mortality in all the treated groups was identical to that in the controls.
Overall assessment	The study investigates the acute sublethal effects of acetamiprid and thiamethoxam and is considered of limited reliability as no analytical verification of the test item was performed, test conditions were not well-defined and the dose-response was non-linear. In addition the study is not performed to a recognised guideline.

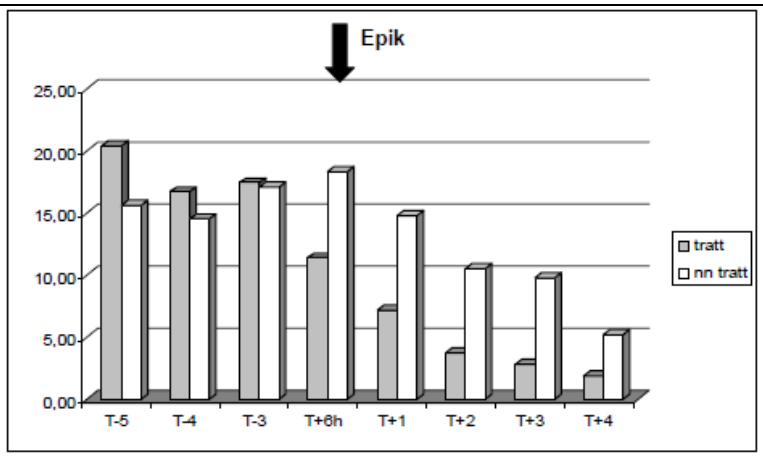
Assessment of levels of repellencey and toxicity of neonicotinoid insecticides on *Apis mellifera liguistica*			
KCA 8.3.2			
Author(s)	Fanti, M., Maines, R., Angeli, G.		
Year	2006		
Journal	ATTI Plant Pathology Days Vol. 1, pp. 51-58		
Relevance check	Relevant		
Reliability check	Reliable: 2 (Klimisch et al., 2007)		
Reasons for no reliability	Not applicable		
Summary	A semi-field trail was performed to evaluate the side-effects of neonicotinoids on *Apis mellifera* L. Five tunnels, including one for the control, of 19.8 m^2 width were sown with *Phacelia tanacetifolia* crop. Each tunnel was divided into six plots of 3.3 m^2 and one hive of 7000 ± 500 bees was positioned inside some days before the spray. The investigations were conducted applying one spray per treatment during bloom on three of the six plots in a randomised design. The parameters assessed were the repellency of foraging honeybees, mortality and health condition of broods. The investigation showed a relevant difference of the selectivity level of the neonicotinoids.		
Reliability check: study details			
Parameter	**Information available**		
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Toxicity tests were carried out following EPPO/OEPP guidelines (1992) ‘Guidelines on test methods for evaluating the side-effects of Plant Protection Products on Honeybees’.		
Test substance Identification of test substance, source, purity, stability	**Trade Name**	**Active Ingredient**	**Composition (% a.i.)**
	Control	Water	-
	Epic	Acetamiprid	20
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity,	No test conditions are reported, however the tests were carried on in semi-field plots (small constructed field plots) subjected to environmental conditions		

<table>
<tr><td>photoperiod, light intensity, number of animals, food availability</td><td></td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Field plots (6.6 x 3 x 2.2 m tunnels) were constructed and planted with Phacelia tanacetifolia. Each constructed plot was covered with aphid-proof netting and subdivided into six portions of 3.3 m^2. Three portions were treated with insecticide and areas left untreated. Five days before insecticide treatments, a colony of bees (7000 ± 5000 bees) were introduced into the tunnels and mortality was monitored daily. During treatment, organisms were not allowed outside of the hive and were kept there for 2 h before being released into the tunnels. Mortality and foraging behaviour was monitored for 5 days before treatment and 4 days after treatment.

<table>
<tr><th>Trade Name</th><th>Dose (ml/hl)</th></tr>
<tr><td>Epic</td><td>37.5</td></tr>
</table>
</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Apis mellifera liguistica were obtained from IASMA aviary. Ages of the organisms’ are not reported.</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>No statistical methods reported</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>For acetamiprid a repellency action on foragers was observed despite the treated and untreated areas not showing a significant difference. The foraging activity showed less of a decrease than thiamethoxam and thiacloprid.

Table 1: Foraging activity on treated areas (AT) and untreated areas (ANT) of each treatment
<table>
<tr><td rowspan="2">Treatments</td><td colspan="3">Pre-treatment foraging</td><td colspan="3">Post-treatment foraging</td></tr>
<tr><td>ANT foraging</td><td>AT foraging</td><td>P (T-test)</td><td>ANT foraging</td><td>AT foraging</td><td>P (T-test)</td></tr>
<tr><td>Epic</td><td>15.73</td><td>18.22</td><td>0.1407</td><td>8.57</td><td>5.40</td><td>0.0568</td></tr>
</table>

Figure 1: Foraging activity on phacelia flowers with Epik treatments in treated and untreated areas</td></tr>
</table>

	
Overall assessment	Due to two key deficiencies, this article should be considered with "limited reliability": • There is no analytical data to support the concentrations tested. • Not complete reporting of crucial methods and data analysis

***Amblyseius andersoni* (Chant) – A new alternative to combat spider mites**	
KCA 8.3.2	
Author(s)	Fiedler, Z.
Year	2009
Journal	Progress in Plant Protection Vol. 49(3), pp. 1469-1473
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this research were to establish the efficacy of *Amblyseius andersoni* in eliminating the pest species the two-spotted spider mite and to assess the toxicity of the insecticides to *A. andersoni*. *A. andersoni* was effective in control of *T. urticae* larvae and adults in ratio 1:2 (predator:pest). The most toxic insecticide to *A. andersoni* was Mospilan 20 SP (acetamiprid) causing 100% mortality at tested concentrations.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Mospilan 20 SP
Test conditions Temperature, pH,	No test conditions were reported. 10 *A. andersoni* were used per replicate, 5 replicates per treatment.

<table>
<tr><td>oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td></td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: sterile water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Predatory Efficacy Experiments: Experiments were conducted in Petri dishes on leaves of cucumber. In each test chamber, 10 individuals of the pest, the two-spotted spider mite, were placed on the leaf. A. andersoni in varying numbers were applied; 1 individual/dish, 2 individuals/dish, or 5 individuals/dish. Each concentration had 5 replicates, No predators were applied in the control. Observations of mortality were made after 24, 48, 72 and 96 h.

Direct Toxicity Experiments: Three concentrations of each insecticide were applied to cucumber leaves infested with the pest species. Observations were made after 24 and 48 h. Leaves were additionally immersed in test solutions and then A. andersoni were placed on each leaf at different times post spraying (after 1, 3 and 5 days post treatment). Each combination was studied with 5 replicates, controls were sprayed with sterile water and observations of mortality were made 24, 48, 72 and 96 h after the addition of test organisms.

<table>
<tr><th>Pesticide</th><th>Doses Tested</th></tr>
<tr><td>Mospilan 20 SP</td><td>0.5x, x, 1.5x</td></tr>
</table>
x = respective field application rate.</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Amblyseius andersoni, no other information is provided</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>No information provided</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Percentage mortality of T. urticae
<table>
<tr><th rowspan="2">Treatment variants</th><th colspan="3">% mortality</th></tr>
<tr><th>1 A. andersoni individual/ petri dish</th><th>2 A. andersoni individuals/ petri dish</th><th>5 A. andersoni individuals/ petri dish</th></tr>
<tr><td>T. urticae</td><td>54</td><td>88</td><td>96</td></tr>
<tr><td>Control</td><td>6</td><td>4</td><td>2</td></tr>
</table></td></tr>
</table>

Table 2: Direct toxicity of insecticides to *A. andersoni*

Treatment	Dose	Mean mortality after time (h) (%)	
		24 hours	48 hours
Mospilan 20 SP	0.5	36 b	70 c
Mospilan 20 SP	1	78 cd	90 d
Mospilan 20 SP	1.5	82 d	100 d
Control 3	0	2 a	6 a

Values accompanied by the same letter in a column do not differ significantly.
(1) – recommended dose; (0.5) – half recommended dose; (1.5) – one and a half times recommended dose

Mospilan 20 SP was the most toxic, causing 100% mortality after 2 days.

Table 3: Mean mortality of *A. andersoni* depending on introduction time

Treatment	Mean mortality (%)	
	Predator Introduction Time (days)	*A. andersoni*
Mospilan 20 SP	1	76 d
Mospilan 20 SP	3	72 d
Mospilan 20 SP	5	56 c
Control 3	0	2 a

Values accompanied by the same letter in a column do not differ significantly ($p < 0.05$, Tukey's test)
(1) – recommended dose; (0.5) – half recommended dose; (1.5) – one and a half times recommended dose

Overall assessment

Due to two key deficiencies, this article should be considered with "limited reliability":

- There is no analytical data to support the concentrations tested.
- Not complete reporting of crucial methods and data analysis

Mechanism for the differential toxicity of neonicotinoid insecticides in the honey bee, *Apis mellifera*	
KCA 8.3.1	
Author(s)	Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, R.M.
Year	2004
Journal	Crop Protection Vol. 23, pp. 371–378
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Laboratory bioassays were conducted to determine the contact honey

	bee toxicity of commercial and candidate neonicotinoid insecticides, including acetamiprid. The LD50 values for nitro-substituted compounds were more toxic than the cyano-substituted compounds. The LD_{50} value for acetamiprid was 7.1 µg/bee. The acetamiprid metabolites, N-demethyl acetamiprid, 6-chloro-3-pyridylmethanol and 6-chloro-nicotinic acid when applied topically, produced no mortality at 50 µg/bee. Since the metabolites of acetamiprid that were investigated were not toxic, any acetamiprid metabolism that would produce these products would be a detoxification mechanism for the honey bee. These results suggest that P450s are an important mechanism for acetamiprid detoxification and low toxicity to honey bees. When honey bees were placed in cages in forced contact with alfalfa treated with acetamiprid and the, triflumizole, in combination at their maximum recommended application rates, no mortality was detected above that of acetamiprid alone or the control.
Reliability check:	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No protocol cited, but detailed methods are reported.
Test substance Identification of test substance, source, purity, stability	Acetamiprid and metabolites (N-demethyl acetamiprid, IM-2-1; 6-chloro-3-pyridiylmethanol, IM-O; and 6-chloro-nicotinic acid, IC-O) (purity of these compounds >99%). Formulated acetamiprid (NI-25, TADS 1242) was 73.9% acetamiprid.
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	Sugar was available ad libitum to the worker bees during the duration of the laboratory bioassay. After treatment, the test container was incubated at 27±1°C, 50% relative humidity and a photoperiod of 14:10 (light:dark). In the laboratory for the plant bioassays, honey bees were fed in their cages with 50% sucrose in a PVC trough and deionized water from an inverted glass jar with a perforated lid. Bees were maintained at 25 ± 2°C with the relative humidity between 77% and 84% under constant darkness except at the observation intervals.
Controls Positive control, negative control	For the lab experiments, controls received 1 µl of ethanol only. For the plant bioassays, there were two non-treated control plots at two sampling intervals.
Dosing system Exposure (dose, duration, frequency)	**Laboratory bioassays** Older worker bees were placed in a 177 ml plastic cup (10–15 bees/cup) covered with a nylon mesh (0.4 cm holes) held in place with a rubber band. A small hole was made in the centre of the cup bottom and a single Kimwipe was partially extruded through the hole into the inside. The cup was then placed into a reservoir of 20% sucrose in water (wt/vol) so that the Kimwipe was soaked with the solution and sugar was available ad libitum to the worker bees during the duration of the assay.

	The insecticides and synergists were separately dissolved in 100% (absolute) ethanol and dilutions were made to obtain the appropriate dose per bee in 1 µl of the solvent. Topical applications to the dorsal thorax of each bee were made with a 50 µl Hamilton syringe fitted with a 1µl repeating dispenser. In all,10 µg of the synergist was applied to anesthetized bees 1 h prior to the insecticide application. The upper limit of the amount of synergist that could be applied was determined by the mortality produced by the most toxic compound (DEF, 29.0% corrected mortality). Corrected percent mortality for the other synergists were as follows: PBO (0%), DEM (0%), triflumizole (0%), epoxiconazole (0%), propiconazole (2.2%), triadimefon (0%) and uniconazole-P (0%). The synergist was applied before the insecticide to provide time for transport of the compound into the insect system and to maximize the likelihood of metabolic inhibition (Zhao et al., 1996 and references therein). Mortality was assessed 24 h after the insecticide treatment in order to minimize control mortality and because in preliminary tests with acetamiprid and nitempyram, only a small or no increase in mortality was found between 24 and 48 h for doses of insecticide that produced mortality in the range of 8–100%. **Plant bioassays** The bioassays consisted of fourteen plots (1 m^2 each) with each plot separated by a 0.6 m buffer zone on all sides. Six plots were treated with acetamiprid (three replicates at two sampling intervals after treatment), another six plots received a combination of acetamiprid and triflumizole (Procure®) (three replicates for two sampling intervals) and there were two non-treated control plots at two sampling intervals. Control and treated plots were harvested at 3 and 24 h after the insecticide application. Acetamiprid and acetamiprid/triflumizole in combination were sprayed on alfalfa at the rate of 168.1 g acetamiprid/ha and 280.2 g triflumizole/ha using a carbon dioxide pressurized (20 psi) handheld sprayer with a single nozzle and a 250 ml tank. The appropriate weight of active ingredient in water was added to the tank and dispensed evenly to an individual plot. Plots were covered after spraying and as needed before rain to prevent insecticide runoff. After application, alfalfa was collected at 3 and 24 h after treatment. The control foliage was collected first, followed by the acetamiprid treatment and then the acetamiprid/ triflumizole treatment. At each sampling interval, 32–72 plants were collected from each treatment and 87–115 plants from the control. 15 g of plants selected at random from the three plots for each treatment and 15 g from a single control plot were placed separately in cages containing 25 bees per cage.

	Test cages were 13x13x13 cm^3 constructed from sheet PVC covered with polyester mesh (3.5 mm mesh).
Test species Body weight or length, gender, age/life stage, source	Honey bees, *Apis mellifera* Linnaeus were collected from two hives on the North Carolina State University Campus (Raleigh, NC); older worker bees were used for the laboratory study. For the plant bioassay, bees were purchased from Apiary Services Inc. (Wareham, MA); worker bees <7 days old were used.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	**Laboratory bioassays** 10–15 bees/cup for laboratory experiments. Each experiment was replicated 2–3 times per dose with a minimum of 30 insects per replicate and 5–7 doses used to determine the LD_{50}. All results were corrected as appropriate for control mortality and/or mortality due to the application of the synergist. **Plant bioassays** Assays were arranged in a randomized block design. Six plots were treated with acetamiprid (three replicates at two sampling intervals after treatment), another six plots received a combination of acetamiprid and triflumizole (Procure®) (three replicates for two sampling intervals) and there were two non-treated control plots at two sampling intervals. The studies were replicated six times at both the 3 and 24 h post-insecticide treatment intervals. **Statistical analysis** Abbot's correction (Abbott, 1925) was applied to all data from dose–response experiments. LD_{50} values were estimated by plotting log dose versus probit plus five mortality (Sokal and Rohlf, 1995; Finney, 1971; Microsoft Excel, 1997). Confidence intervals for toxicity ratios were determined by the method of Robertson and Preisler (1992). Means tests were conducted using Student's t-test (P<0.05).
Biological effects Determined effect concentration, dose response observed	**Table 1: Mortality 24 h after topical application** | | **LD_{50} (µg/bee)** | | Acetamiprid | 7.07 | The LD_{50} value for acetamiprid was 7.1 µg/bee (465 insects tested; 95% CI 4.57-11.2. The major plant metabolites of acetamiprid are IM-2-1, IM-O and IC-O (Tokieda et al.,1997). They were not highly toxic when applied topically. At a dose of 50 µg/bee, no mortality was found above that of the control for bees treated with IM-2-1, IM-O and IC-O. Since the metabolites were non-toxic, any metabolism that would produce these products would be a detoxification mechanism for the honey bee.

	The measurement of acetamiprid toxicity for insects pretreated with different metabolic inhibitors found no significant increase in acetamiprid toxicity for bees pretreated with 10 µg of DEF or DEM. However, a statistically significant synergistic ratio of 2.96 was found for DEF. PBO, uniconazole-P and DMI-fungicides had a much greater effect on acetamiprid toxicity than DEF or DEM. In the plant bioassays, average mortality for plants treated with acetamiprid and triflumizole in combination was 4% at 3 h after application. This was not significantly different from that obtained by acetamiprid alone or the control. At 24 h after treatment, the average mortality with this combination was 2% which was again not significantly different from acetamiprid alone or the control.
Overall assessment	The study investigated the effects of neonicotinoids on the honey bee and is considered to be reliable with analytical verification of the test material and well-defined test conditions. The study was not performed in line with a recognised guideline.

Residual toxicity of pymetrozine, spiromesifen, spinosad and acetamiprid to the predacious ladybird *Serangium parcesetosum* (Coleoptera: Coccinellidae), a predator of the whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) on greenhouse crops in the East Mediterranean region of Turkey	
KCA 8.3.2	
Author(s)	Kutuk, H. Yigit, A.
Year	2009
Journal	IOBC/wprs Bulletin Vol. 49, pp. 353-358
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this study were to determine the toxicity of five pesticides, including acetamiprid, to *Serangium parcesetosum* using the IOBC approach. To evaluate residual contact activity, *S. parcesetosum* were placed in treated petri dishes and their mortality was monitored daily for 5 days. The fecundity of surviving females were tested for 15 days and egg fecundity and hatching were recorded. Acetamiprid was detrimental by contact to both stages of the test organism at the recommended application rates for the control of thrips and whiteflies, respectively.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Toxicity tests were carried out with modified methods defined by the IOBC (Schmuck et al., 2000).
Test substance Identification of test substance, source,	Acetamiprid 20SC

<table>
<tr><td>purity, stability</td><td></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>25 ± 1ºC, 70 ± 10% relative humidity and 16:8 h light:dark photoperiod. Test organisms were given access to leaves infested with B. tabaci as food.</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: tap water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Glass petri dishes (9 x 1.7 cm) were sprayed with a hand sprayer to obtain 2 mg of wet deposit per cm^2 for each pesticide or tap water (as control). They were left to dry for 2-3 h before 10 S. parcesetosum 3-5 day old larvae were added to the test chambers. Dead larvae were recorded daily and the total larval mortality was calculated after 5 days.

<table>
<tr><th>Compound</th><th>Dose</th></tr>
<tr><td>Acetamiprid 20SC</td><td>0.3 kg/ha</td></tr>
</table>

Adults that emerged were tested for fecundity. Groups of ten adults were transferred into clean petri dishes and eggs laid on the leaf with the food source were counted daily. The eggs were stored under the same environmental conditions until larval hatching. Fecundity was assessed over a total of 14 days. Adult sex was determined at the end of the fecundity assessment and daily egg load per female was calculated.

The same protocol was followed with ladybird adults.</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Adult S. parcesetosum were collected from a citrus grove in Hatay, Turkey and cultured following methods of Yigit (1992). 3-5 day old larvae were used in experiments.</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Ten organisms were used per replicate with at least three replicates per pesticide for larval and adult toxicity and fecundity experiments. Forty replicates were performed for each pesticide and developmental stage tested.

Mortality was calculated by the following equation:

E = 100% - (100% - M)*R

Where M = percent mortality calculated by Abbott’s formula, R = ratio between mean number eggs laid in pesticide treatments and in control.</td></tr>
</table>

<table>
<tr><td></td><td>Pesticides were classified according to IOBC evaluation categories: 1) harmless, $E < 30\%$; 2) slightly harmful, $30\% < E < 80\%$; 3) Moderately harmful, $80\% < E < 99\%$; 4) Harmful, $E > 99\%$</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Acetamiprid was highly toxic, caused total mortality and was classified as Class 4 for all stages of the ladybird.

Table 1: Pesticide side effects on S. parcesetosum larvae
<table>
<tr><th rowspan="2">Pesticide</th><th colspan="5">Exposed as Larvae</th></tr>
<tr><th>No. dead larvae</th><th>No. survivors</th><th>Emerged adults</th><th>Mean egg/female/day</th><th>Class</th></tr>
<tr><td>Acetamiprid 20SC</td><td>40</td><td>0</td><td>0</td><td>--</td><td>4</td></tr>
<tr><td>Control</td><td>2</td><td>38</td><td>38</td><td>3.1</td><td>--</td></tr>
</table>
Table 2: Pesticide side effects on S. parcesetosum adults
<table>
<tr><th rowspan="2">Pesticide</th><th colspan="4">Exposed as Adults</th></tr>
<tr><th>No. dead adults</th><th>No. survivors</th><th>Mean egg/female/day</th><th>Class</th></tr>
<tr><td>Acetamiprid 20SC</td><td>40</td><td>0</td><td>-</td><td>4</td></tr>
<tr><td>Control</td><td>0</td><td>40</td><td>3.0</td><td>-</td></tr>
</table></td></tr>
<tr><td>Overall assessment</td><td>Due to key deficiencies, this article should be considered with ‘limited reliability’:
There is no analytical data to support the concentrations tested.
A dilution series was not used in exposures, only 1 concentration (the recommended field application rate) was used.
Not complete reporting of some methods</td></tr>
</table>

Evaluation of chronic toxicity of four neonicotinoids to *Adalia bipunctatta* L. (Coleoptera: Coccinellidae) using a demographic approach	
KCA 8.3.2	
Author(s)	Lanzoni, A., Sangiorgi, L., Luigi, V. de, Consolini, L., Pasqualini, E., Burgio, G.
Year	2012
Journal	IOBC WPRS Bulletin Vol. 74, pp. 211-217
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this study were to utilize demographic and population modelling for estimation of pesticide effects on *Adalia bipunctatta*. Bioassays were carried out in the laboratory to assess the demographic responses of *A. bipunctatta*, exposed as larvae or adults, to neonicotinoid insecticides at sublethal doses. Demographic parameters were calculated by means of life tables. Life table data were also used to generate an age-classified projection model. The

<table>
<tr><td></td><td>elasticity of population growth rate to change and the Delay in Population Growth Index were also calculated. Exposure of larval stage to acetamiprid significantly reduced all demographic parameters in comparison with control with the exception of mean generation time and results in a pronounced slower increase in the population. For all insecticides tested, the perturbation analysis showed that survival, in particular larval stages, had the greatest effect on population growth. Neonicotinoids caused significant population delays with a more pronounced effect when adults were exposed.</td></tr>
<tr><td colspan="2">Reliability check: study details</td></tr>
<tr><td>Parameter</td><td>Information available</td></tr>
<tr><td>Test protocol
GLP, GEP, Guidelines (US EPA, OECD, …)</td><td>No specific protocol is cited, but detailed methods are provided</td></tr>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td><table><tr><th>Compound</th><th>Trade Name</th></tr><tr><td>Acetamiprid</td><td>Epik</td></tr></table></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>No test conditions are reported</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative control: water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Laboratory assays were carried on by treating potted peach plants with 1/5 of the recommended field dose of 4 neonicotinoids. Each peach plant was greenhouse-reared, sprayed with treatment which exposed larvae for 24 hours and adults for six hours. For each treatment 15 survived adult females from both larvae and adults were maintained and monitored for 15 days.

<table><tr><th>Compound</th><th>Dose</th></tr><tr><td>Acetamiprid</td><td>5 g/hl</td></tr><tr><td>Negative Control</td><td>water</td></tr></table></td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Adalia bipunctata larvae and adults</td></tr>
<tr><td>Statistical analyses
Sample size/ replicates, statistical analysis of</td><td>Life tables were calculated following methods by Carey (1993). Demographic parameters net reproductive rate (R_o), intrinsic rate of increase (r_m), mean generation time (T), doubling time (DT) and</td></tr>
</table>

data (significance level, variability)

finite rate of increase (λ) were calculated. Jackknife method was used to improve the estimations and to calculate the variability of parameters (Maia et al., 2000).

Life table data were also used to generate an age-classified Leslie projection matrix (Caswell, 2001) to model the impact that exposure would have on a population. The model consists of a matrix including survival probabilities (*Pi*) and fertilities (*Fi*) of a population. This matrix was multiplied with a starting population vector that contains information on the age distribution of the studied population. Population growth across time can then be found via repeated matrix multiplications.

Perturbation analysis was utilised to calculate the elasticity of population growth rate to change in each of the individual vital traits *Pi* and *Fi* (Caswell, 2001). Total value of *Pi* and *Fi* elasticity are presented. Finally, an application of matrix models, the Delay in Population Growth Index (Wennergren and Stark, 2000), a measure of population recovery, was calculated to compare the time required to control a population and pesticide-exposed populations to reach a predetermined number of individuals.

Biological effects
Determined effect concentration, dose response observed

Exposure of larval stage to acetamiprid significantly reduced all demographic parameters in comparison to the control with the exception of mean generation time (T).

Table 1: Life table parameters for *A. bipunctata* exposed as larvae (mean ± standard deviation)

Treatment	R_o	T	r_m	DT	λ
Control	105.85 ± 15.16a	26.99 ± 0.15a	0.1727 ± 0.0053a	4.013 ± 0.124a	1.1885 ± 0.0063a
Acetamiprid	37.41 ± 9.31b	27.39 ± 0.33a	0.1322 ± 0.0102b	5.242 ± 0.415b	1.1414 ± 0.0116b

Means within column followed by the same letter were not statistically different (Kruskal-Wallis test; $p < 0.05$)

Table 2: Life table parameters for *A. bipunctata* exposed as adults (mean ± standard deviation)

Treatment	R_o	T	r_m	DT	λ
Control	65.81 ± 13.62a	27.57 ± 0.47ac	0.1518 ± 0.0081a	4.565 ± 0.247a	1.1640 ± 0.009 4a
Acetamiprid	43.54 ± 14.91ac	28.13 ± 0.59ac	0.1342 ± 0.0140ab	5.166 ± 0.564ab	1.1436 ± 0.0159ab

Means within column followed by the same letter were not statistically different (Kruskal-Wallis test; $p < 0.05$).

Larval exposure to acetamiprid results in a pronounced slower increase in the coccinellid population. Contributions of survivorship and fertility to population growth rate were not different among treatments. For all insecticides tested, the contribution of survival (*Pi*) to the population growth rate was higher than that of fertility (*Fi*) underlining that survival, in particular of larval and imaginal stages

	and not reproduction, had the greatest effect on population growth. **Table 3: Elasticity of population growth rate as a function of sublethal concentrations of neonicotinoids.** **Control** / **Acetamiprid** **Exposed as larvae** *Pi*: 0.9609 / 0.9617 *Fi*: 0.0391 / 0.0383 **Exposed as adults** *Pi*: 0.9613 / 09621 *Fi*: 0.0387 / 0.0379 The values of delay in population growth index showed that neonicotinoids caused significant population delays with a more pronounced effect in exposed adults. This is in agreement with the higher reproductive values seed in adults compared to larvae.
Overall assessment	Due to key deficiencies, this article should be considered with 'limited reliability': • There is no analytical data to support the concentrations tested. • There was not a dilution series used in toxicity tests, only 1/5 the recommended field application rate. • Not complete reporting of key methods and data analysis • Study not performed in line with recognised guideline

Laboratory studies to elucidate the residual toxicity of eight insecticides to *Anystis baccarum* (Acari: Anystidae)	
KCA 8.3.2	
Author(s)	Laurin, M-C., Bostanian, N.J.
Year	2007
Journal	Journal of Economic Entomology Vol. 100(4), pp. 1210-1214
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	Study determined toxicity of methoxyfenozide, acetamiprid, thiamethoxam, imidacloprid, spinosad, phosmet, carbaryl and λ-cyhalothrin pesticides to *Anystis baccarum* a common predatory mite in apple orchards and vineyards. Methoxyfenozide, acetamiprid, thiamethoxam, imidacloprid and spinosad were found to be nontoxic in 48 hr petri-dish residue tests. Lambda-cyhalothrin, phosmet and carbaryl residues were toxic in 48 hr petri-dish residue tests. LC50 values were greater than field application rates for these pesticides.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No specific protocol is cited. Detailed methods are provided in the Materials and Methods section.
Test substance Identification of test	Acetamiprid (Assail 70 WP)

<table>
<tr><td>substance, source, purity, stability</td><td></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>• Temperature: 21C
• Relative humidity: 80%
• Organisms were not fed during test
• 16:8 light:dark photoperiod</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>A negative control was used, but what served as a control was not reported</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Pesticide was applied to the interior, side walls and covers of plastic petri dishes (50 x 9 mm) with a residue of 2 mg/cm^2.

Insecticide Dosing Regime
Insecticide | Application rate in Orchards (g a.i./L) | Range of Concentrations (g a.i./L)
Acetamiprid | 0.1543 | 0.1543 (X) – 2.4688 (16X)
X = g a.i./L based on the application of 1,000 L of sprayable material per hectare.</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Anystis baccarum (predacious mite) adults, field collected from Apple orchard foliage. No gender or size classification</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Two (non-toxic insecticides) or three (toxic insecticides) replicates with 30 mites per replicate for a total of 60-90 mites per replicate.

Probit analyses carried out with Polo PC software. Mortality corrected according to Abbott (1925).</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Average Percentage of Mortality
Insecticide | Mortality (Concentration)
Acetamiprid | 0.0 (16X), 0.0 (8X), 3.7 (4X), 0.0 (2X), 0.0 (X), 3.3 (Control)
X = g/L (a.i.) based on 1000 L of sprayable material per hectare.</td></tr>
<tr><td>Overall assessment</td><td>Methodology, results and discussion were well documented. However, the study is considered slightly reliable due to the following concerns:
• Negative controls were not described
• No size or gender classification for the organisms used
• Organisms were field collected from an orchard and it is unknown if they were compromised in any way.
• There was no chemical analysis done to determine concentrations used.
• Study not performed in line with recognised guideline.</td></tr>
</table>

Toxicity of neonicotinoid insecticides to honey bees: Laboratory tests

<table>
<tr><td colspan="2">KCA 8.3.1</td></tr>
<tr><td>Author(s)</td><td>Laurino, D., Porporato, M., Patetta, A., Manino, A.</td></tr>
<tr><td>Year</td><td>2011</td></tr>
<tr><td>Journal</td><td>Bulletin of Insectology Vol. 64(1), pp. 107-113</td></tr>
<tr><td>Relevance check</td><td>Relevant</td></tr>
<tr><td>Reliability check</td><td>Reliable: 1 (Klimisch et al., 2007)</td></tr>
<tr><td>Reasons for no reliability</td><td>Not applicable</td></tr>
<tr><td>Summary</td><td>Feeding and contact toxicity study of insecticides thiamethoxam, clothianidin, acetamiprid and thiacloprid to Apis mellifera (honey bee). Acute oral toxicity tests were conducted dispersing commercial formulation in sugar syrup, contact toxicity tests were conducted with treated Spanish chestnut leaves. An acute oral lethal dose (LD50), the acute indirect contact Lethal Concentration$_{50}$ and hazard quotient were calculated at 24, 48 and 72 hrs. Acetamiprid caused higher mortality then untreated controls only in oral toxicity tests when honey bees were starved prior to exposure. Honey bees that died during tests were analysed for insecticide residues.</td></tr>
<tr><td colspan="2">Reliability check: study details</td></tr>
<tr><td>Parameter</td><td>Information available</td></tr>
<tr><td>Test protocol
GLP, GEP, Guidelines (US EPA, OECD, …)</td><td>Modified OEPP/EPPO (2003) methods as reported by Arzone and Vidano (1980). Modifications were made by repeatedly monitoring bee behaviour during ingestion tests and indirect contact tests in place of topic contact tests.</td></tr>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td>
<table>
<tr><th>Active Ingredient</th><th>Trade Name</th><th>Formulation</th><th>% a.i.</th></tr>
<tr><td>Acetamiprid</td><td>Epik</td><td>Soluble powder</td><td>5% w/w</td></tr>
</table>
</td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>Test conditions were not reported, however, a citation was used (Laurino et al., 2010)</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative control: Water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Acute oral toxicity tests were conducted following Laurino et al. (2010). Ingestion tests after starvation followed this procedure except the bees were starved for two hours after capture.

Indirect contact tests were conducted by treating Spanish chestnut leaves by spraying to drip with hand sprayer and left to dry for 3 hrs prior to introducing them into test chambers. Ten bees were added to test chambers lined with treated leaves with three replicates. Test duration was 72 hrs.</td></tr>
</table>

<table>
<tr><td></td><td>
<table>
<tr><th>Test Type</th><th>Compound</th><th>Dose Range</th></tr>
<tr><td>Ingestion</td><td>Acetamiprid</td><td>100 ppm</td></tr>
<tr><td>Indirect Contact</td><td>Acetamiprid</td><td>100 ppm</td></tr>
<tr><td>Ingestion after Starvation</td><td>Acetamiprid</td><td>100, 50, 20 ppm</td></tr>
</table>
</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Apis mellifera, no other information is reported on test species</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>For each test material at each concentration and the controls, 30 bees were used.

The number of dead and live honey bees was compared with the control group by the Fisher exact test. If statistically significant differences were not detected, 30 other honey bees underwent testing and the resulting mortality pooled with the previous one. The chi-square test was performed on the resulting 60 organisms and controls. The LC50 both by ingestion and indirect contact was calculated by means of logit analysis.

The amount of syrup ingested by each honey bee during oral toxicity test had previously been determined by weighing the feeder at the beginning and end of the allowed one hr feeding period. The ingestion lethal dose (LD50) was obtained from the relative LC50. LD50 were used to calculate the Hazard Quotient: HQ = field application rate (g/ha)/(oral LD50 (µg/bee)).

LD50 and HQ could not be calculated for indirect contact tests because the absorbed amount could not be determined.</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>During the trials, the honey bees showed obvious symptoms of poisoning, such as shaking and tremors, uncoordinated and uncontrolled movements, staggering, inability to take up a correct position of the body and prolonged frenetic movement of the legs and rotation when in the supine position. Direct observation of the behaviour of the honey bees in cages proved that it was transitory for acetamiprid at field concentration.

Ingestion tests
Acetamiprid showed no mortality in the ingestion tests even 72 h from test initiation.

Indirect contact tests
Acetamiprid showed no mortality in the indirect contact tests even 72 h from test initiation.

Ingestion tests after starvation
The mortality caused by acetamiprid was 50.85% at 100 ppm.</td></tr>
</table>

	Statistically significant mortality was observed at 50 ppm 72 h from test initiation. The mortality caused by thiacloprid was not total even 72 h from test initiation, but resulted statistically significant up to the concentration of 36 ppm.
Overall assessment	Methodology, results and discussion were well documented. The study is considered very reliable.

Comparative toxicity of imidacloprid and its transformation product 6-chloronicotinic acid to non-target organisms: Microalgae *Desmodesmus subspicatus* and amphipod *Gammarus fossarum*	
KCA 8.2.4	
Author(s)	Malev, O., Klobučar, R.S., Fabbretti, E., Trebše, P.
Year	2012
Journal	Pesticide Biochemistry and Physiology Vol. 104, pp. 178-186
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of the study were to determine physiological/ biochemical biomarkers, behavioural alterations and mortality to *Gammarus fossarum* and growth inhibition of *Desmodesmus subspicatus* from exposure to imidacloprid, the IMI commercial mixture Confidor 200SL and the IMI transformation product 6-chloronicotinic acid. Algal growth has shown significant sensitivity to Confidor 200SL and 6-chloronicotinic acid when compared to imidacloprid. In *G. fossarum* low doses of imidacloprid (102.2 µg/L) were sufficient to induce lipid peroxidation, while Confidor 200SL induced increased catalase activity (511.3 µg/L) and lipid peroxidation (255.6 µg/L). 6-chloronicotinic acid altered catalase activity without changing lipid peroxidation.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No specific protocols cited, however methods are well detailed and reported.
Test substance Identification of test substance, source, purity, stability	<table><tr><th>Compound</th><th>Trade Name</th><th>Purity</th></tr><tr><td>Imidacloprid</td><td>Pestanal®</td><td>99.8%</td></tr><tr><td>Imidacloprid</td><td>Confidor 200SL</td><td>200 g a.i./L</td></tr><tr><td>6-chloronicotinic acid</td><td>--</td><td>97%</td></tr></table> Imidacloprid and 6-chloronicotinic acid stability was confirmed by measuring the concentrations of the substances at the beginning and end of the study.
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity,	Algae Toxicity Tests: 23 ± 1 ºC, light intensity 1100 lux Gammarus Toxicity tests: 12 ± 2 ºC, 60% relative humidity and kept in dark. Temperature, pH, conductivity and dissolved oxygen were monitored throughout experiments.

<table>
<tr><td>photoperiod, light intensity, number of animals, food availability</td><td></td></tr>
<tr><td>Controls
Positive control, negative control</td><td>• Algae Negative control: co-formulants (38.4% demthylsulfoxide, 37.5% 1-methyl-2-pyrrolidone and 24.1% deionised water.
• Algae Positive Control: Potassium dichromate
• Gammarus negative control: co-formulants</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Algae toxicity test
Algae chronic toxicity tests were carried out in 96 microwell plates. The algal inoculum was taken from an exponentially growing pre-culture and added into 25 mL of growth media in order to obtain an initial cell density of 104 cells/mL. 200 µl of test solution was added to wells. Serial dilutions of tested pesticides were made in algae medium, six replicates of controls and three replicates of each test concentration were applied. All plates were incubated for 4 days.

The following range of equal molar concentrations was prepared for all tested compounds: 7.6; 25.6; 51.1; 127.8 and 255.6 mg/L for imidacloprid and 4.7; 15.7; 31.5; 78.7 and 157.5 mg/L for 6-chloronicotinic acid. For Confidor 200SL the final concentrations were corresponding to 0.003–0.12% (v/v) which contained 7.6–255.6 mg/L of imidacloprid. Lower concentrations of imidacloprid than those monitored in this experimental trial were already tested on D. subspicatus and showed no effect on algal growth up to 10 mg/L and due to this fact were excluded.

Amiphods toxicity test
Gammarids were exposed for 24 hr. Toxicity tests were carried out in plastic petri dishes with 50 individuals per exposure concentration.

Table 1: Test concentrations
<table>
<tr><th>Toxicity Test</th><th>Compound</th><th>Concentration (mg/L)</th></tr>
<tr><td rowspan="10">G. fossarum</td><td rowspan="5">Imidacloprid and Confidor 200SL</td><td>102.2</td></tr>
<tr><td>153.3</td></tr>
<tr><td>204.5</td></tr>
<tr><td>255.6</td></tr>
<tr><td>511.3</td></tr>
<tr><td rowspan="5">6-chloronicotinic acid</td><td>62.8</td></tr>
<tr><td>94.6</td></tr>
<tr><td>126.2</td></tr>
<tr><td>157.7</td></tr>
<tr><td>315.5</td></tr>
<tr><td rowspan="3">D. subspicatus</td><td rowspan="3">Imidacloprid and Confidor 200SL</td><td>7.6</td></tr>
<tr><td>25.6</td></tr>
<tr><td>51.1</td></tr>
</table></td></tr>
</table>

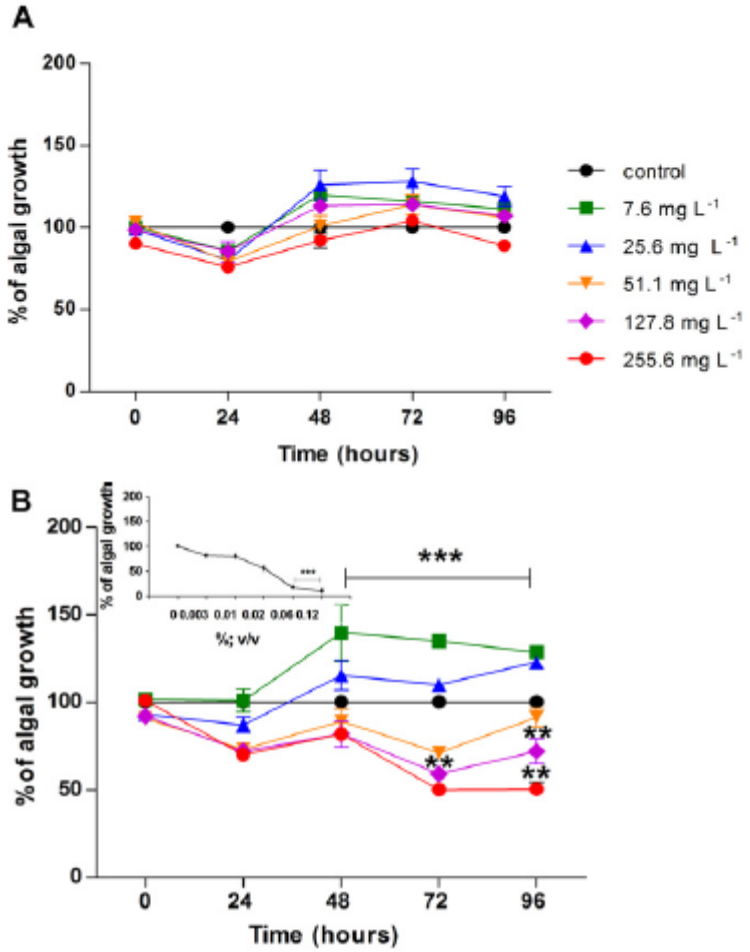
			127.8
			255.6
		6-chloronicotinic acid	4.7
			15.7
			31.5
			78.7
			157.5

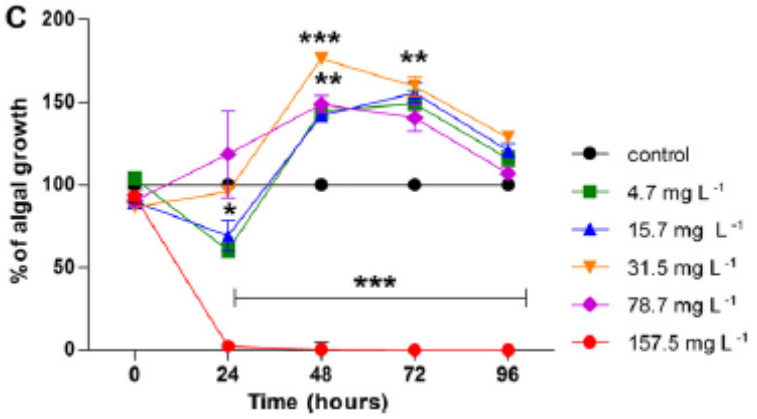
Biochemical biomarker assays
Activity of acethylcholinesterase (AChE) was determined using DTNB and acetylthiocoline iodide as substrate according to Ellman et al. (1961). CAT activity was determined according to the method of Jamnik and Raspor (2003). GST activity was determined according to the protocol of Habig et al (1974). All the data relative to the enzymatic activity are normalised to the total protein content based on the method of Bradford. LP was estimated in vitro after the formation of malondialdehyde (MDA), a major by-product of lipid peroxidation that reacts with thiobarbituric acid, with slight modifications.

Test species Body weight or length, gender, age/life stage, source	Algae Toxicity Tests: *Desmodesmus subspicatus* from Helmholtz Centre for Environment Research-UFZ, Leipzig grown in laboratory following ISO methods. Gammarus Toxicity Tests: *Gammarus fossarum* were field collected from stream Vogršček, Slovenia. Organisms were kept in 20 L glass aquaria at 12 ± 2ºC, 8:16 hr light:dark photoperiod for at least 14 days prior to testing. Organisms used for testing were adult males, total body length and total wet weight was measured prior to testing.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	All statistical tests were performed using STATISTICA 7 StatSoft software. Comparisons were conducted between control and exposure data using the Student's t-test or the Mann-Whitney rank sum test after the software direct choice of parametric or nonparametric data. Multiple comparison were analysed with the one-way ANOVA and Tukey post-test
Biological effects Determined effect concentration, dose response observed	**Algae toxicity test** Algal chronic toxicity revealed a high toxic potential of 6-chloronicotinic acid at the highest concentration (Fig. 1C). 6-chloronicotinic acid induced some perceivable alterations in algae growth, causing slight and temporary inhibition effects at lower doses (4.7 and 15.7 mg/L already after 24 h compared to control ($p < 0.05$) (Fig. 1C). The highest dose of 6-chloronicotinic acid extensively suppressed the algal growth Overall 6-chloronicotinic acid effects were stimulatory on algae growth. Major stimulatory effect of 6-chloronicotinic acid was observed at 31.5 mg/L (48 h) reaching 176.4 ± 3.4% and stayed significantly increased also after 72 h compared to control ($p < 0.001$) (Fig. 1C). It was not possible to calculate the IC50 value for imidacloprid due to its low inhibitory effects within the entire range of tested concentrations (Fig. 1A).

Furthermore, the toxicity of Confidor 200SL ranged from 27.9% up to 49.72% (Fig. 1B). Inhibition of algal growth was significant at 127.8 and 255.6 mg/L compared to control ($p < 0.01$). Higher toxicity of Confidor 200SL was possibly induced by the co-formulants present in the commercial formulation which contributed as a major part to toxicity for algae. The co-formulants alone induced a significant inhibition of 82.3% and 89.7% (at 0.06 and 0.12%; v/v) compared to control ($p < 0.001$) (Fig. 1B).

Figure 1: *D. subspicatus* % of algal growth compared to control to imidacloprid (A), Confidor 200SL (B) and 6-chloronicotinic acid (C) at 24, 48, 72 and 96 hr. The inside graph of B represents negative control co-formulants only. Concentration for (A) is the same for (B).





Amphipods toxicity test

All specimens presented a mean total body length of 12.35 ±0.25 mm and mean weight of 0.029 ± 0.002 g. The negative control (co-formulants mixture) did not have any adverse effects on *G. fossarum* at all tested concentrations. Furthermore, concentrations of all tested compounds lower than 102.2 l g/L for imidacloprid and 62.8 l g/L 6-chloronicotinic acid did not induce significant effects compared to control. Our data demonstrated slight toxicity of imidacloprid with minor changes in mortality rate (Fig. 2A). Imidacloprid induced only 22.3% ± 5.09 of dead organisms at 102.2 µg/L. Confidor 200SL demonstrated an increased effect on mortality, especially at higher concentrations. Percentages of dead organisms at 255.6 and 511.3 µg/L of a.i. reached 40 ± 5.7% and 45.5 ± 7.3%, respectively (Fig. 2A). This increased mortality was significant for the both concentrations ($p < 0.05$). On the contrary 6-chloronicotinic acid showed an overall low toxicity, ranging from 8.6 ± 1.9% up to 14.1 ± 1.1% (at 62.8 and 315.5 µg/L, respectively; Fig. 2B). At 511.3 µg/L of imidacloprid and Confidor 200SL was present a high number of inactive animals with only respiration movements. These values were of 76.6 ± 6.6% for imidacloprid and of 90 ± 5.7% for Confidor 200SL ($p < 0.001$; compared to control). Number of moulted amphipods after 24 h exposure to 6-chloronicotinic acid at 315.5 µg/L was of 56.6 ± 3.3% ($p < 0.001$). Number of moulted animals was minor after 24 h of exposure to imidacloprid and Confidor 200SL at 511.3 µg/L (23.3 ± 3.3% and 13.3 ± 3.3%, respectively; ($p > 0.05$). 6-chloronicotinic acid seemed to induce overall hyperactivity and rapid swimming (with numerous sideways and back-and-forth movements) which affected 80 ± 5.7% of total treated gammarids at 315.5 µg/L 6-chloronicotinic acid (compared to control; $p < 0.001$).

Figure 2: Mortality rate of *G. fossarum* after 24 hr exposure to IMI or Confidor 200SL (A) and 6CNA (B)

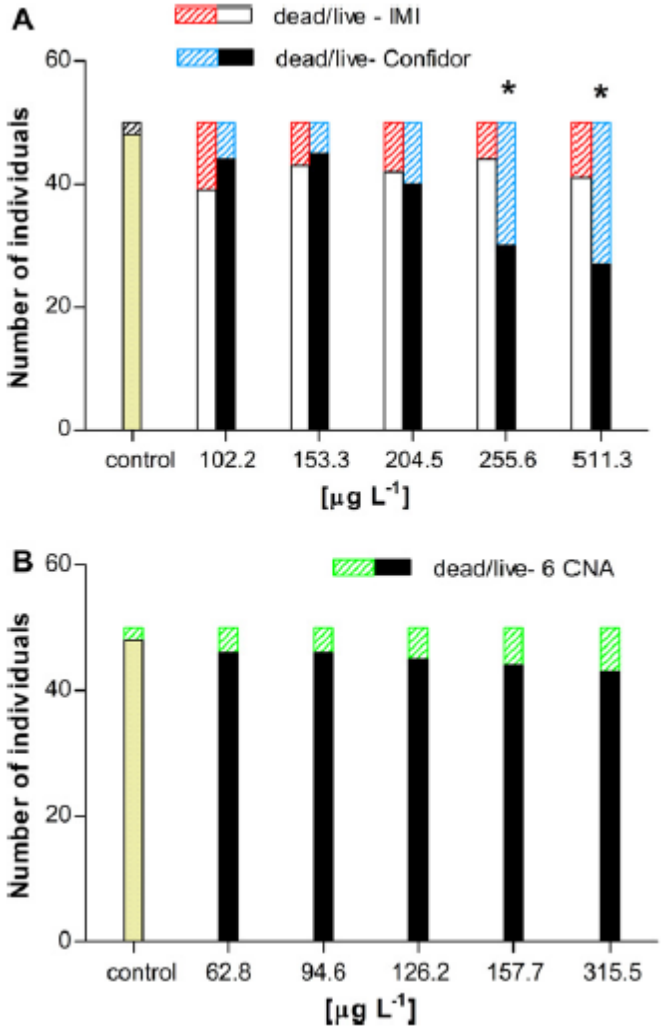


Table 2: Number of immobile/paralysed, hyperactive and moulted individuals of *G. fossarum* exposed to imidacloprid

Compound	Conc.	Immobile Individuals	Hyperactive Individuals	Moulted Individuals

Imidacloprid	Control	None	None for all concentrations	None
	102.2	16.6 ± 3.3		10 ± 5.7
	153.3	16.6 ± 8.8		13.3 ± 3.3
	204.5	13.3 ± 3.3		23.3 ± 8.8
	255.6	43.3 ± 3.3		26.6 ± 3.3
	511.3	76.6 ± 6.6		23.3 ± 3.3
Confidor 200SL	Control	None	None for all concentration	None
	102.2	23.3 ± 3.3		6.6 ± 3.3
	153.3	33.3 ± 3.3		13.3 ± 3.3
	204.5	46.6 ± 14.5		13.3 ± 8.8
	255.6	56.6 ± 3.3		10 ± 0
	511.3	90 ± 5.7		13.3 ± 3.33
6-chloro nicotinic acid	Control	None for all concentrations	None	None
	62.8		16.6 ± 3.3	20 ± 5.7
	94.6		23.3 ± 3.3	33.3 ± 3.3
	126.2		43.3 ± 3.3	43.3 ± 12
	157.7		43.3 ± 3.3	46.6 ± 3.3
	315.5		80 ± 5.7	56.6 ± 3.3

Effects on enzyme activities and lipid peroxidation

G. fossarum exposed to imidacloprid displayed no significant changes of AChE activity at all concentrations. The AChE values at all exposure concentrations of imidacloprid ranged between 70.6 ± 7.8 and 78.2 ± 11.6 µmol/min/mg proteins ($p > 0.05$; compared to control). CAT activity was not modified after imidacloprid exposure (Fig. 3A). The values ranged between 22.04 ± 1.5 µmol/min/mg protein for control and 28.4 ± 8.6 µmol/min/mg protein at 255.6 µg/L. Commercial formulation induced a moderate change in CAT at 511.3 µg/L a.i. going up to 48.06 ± 9.7 µmol/min/mg protein compared to control ($p < 0.05$). Values of CAT activity in the case of exposure to 6-chloronicotninc acid reached 48.9 ± 6.7 µmol/min/mg protein already at 157.7 µg/L ($p < 0.001$) (Fig. 3B). After exposure to Confidor 200SL two different outcomes for GST activity at 255.6 and 511.3 µg/L were evident (Fig. 4A). At 255.6 µg/L was present an observable, but statistically not significant decrease in GST activity ($p = 0.053$). The values of GST went from control values of 419.1 ± 101.8 mmol/min/mg protein to 286.8 ± 92.71 nmol/min/mg protein at 255.6 µg/L. Higher concentration of Confidor 200SL (511.3 µg/L of a.i.) induced an increase of GST activity up to 831.4 ± 117.2 nmol/min/mg protein ($p < 0.05$). Imidacloprid and 6-chloronicotninc acid exposure provoked no significant changes in GST activity compared to control ($p > 0.05$) (Fig. 4A and B, respectively). Imidacloprid induced at 102.2 µg/L an increase in lipid peroxidation (LP) levels (Fig. 5A). This increase was 2.7-fold higher in contrast to the control group ($p < 0.01$). On contrary, Confidor 200SL induced significant rise of thiobarbituric acid reactive substances (TBARS) only at higher dose (255.6 µg/L of a.i.; $p < 0.05$). This increase was lower than the significant peak induced by imidacloprid at 102.2 µg/L (Fig. 5A). No significant effect of 6-chloronicotnic acid on LP increase was noted after 24 h at all concentrations (Fig. 5B). However, it was detected a significant decrease of LP values at

315.5 µg/L ($p < 0.001$).

Figure 3: Whole-body CAT activity (µmol/min/mg protein) of *G. fossarum* measured after 24 h of exposure to IMI or Confidor 200SL (A) and 6 CNA (B). Boxes represent minimum and maximum values and (•) represents mean.

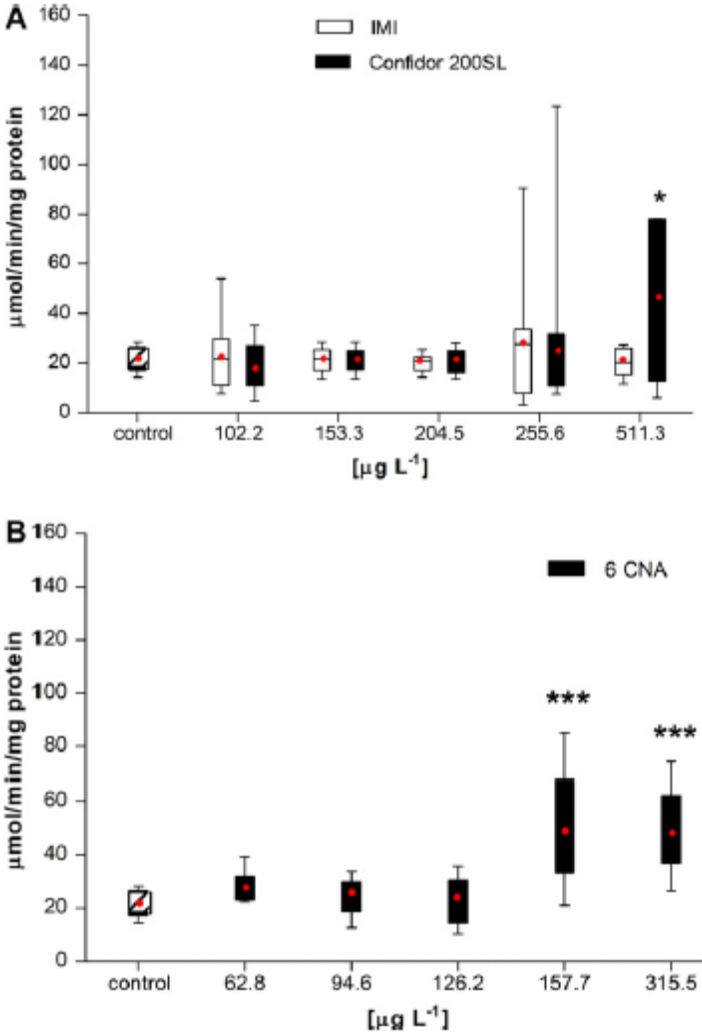


Figure 4: Whole-body GST activity (nmol/min/mg protein) of *G. fossarum* measured after 24 h of exposure to IMI or Confidor 200SL (A) and 6 CNA (B). Boxes represent minimum and maximum values and (•) represents mean.

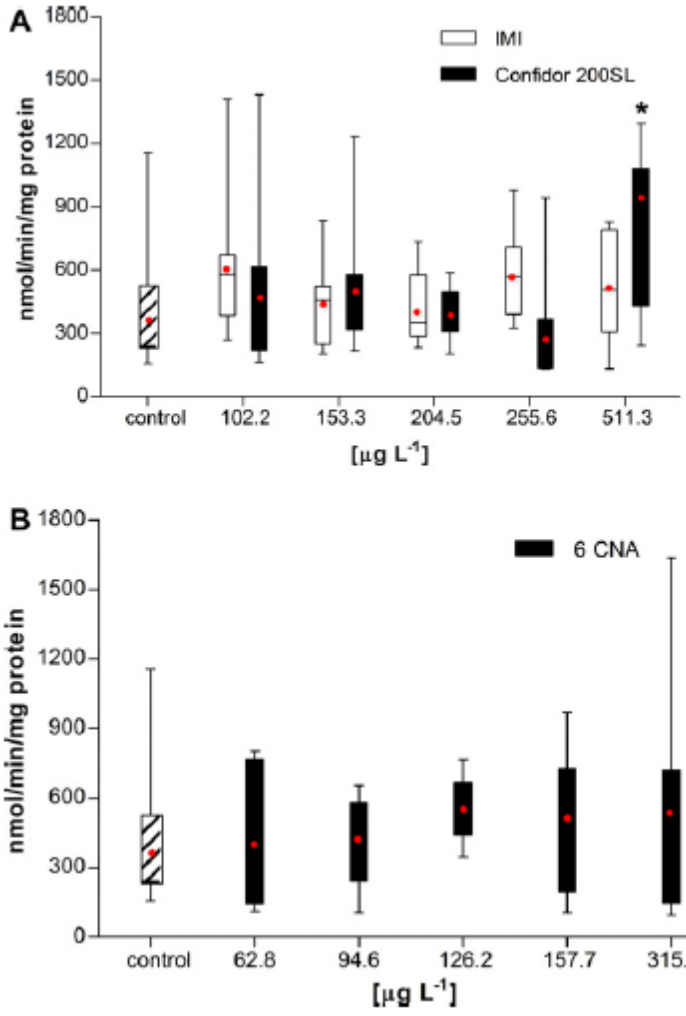
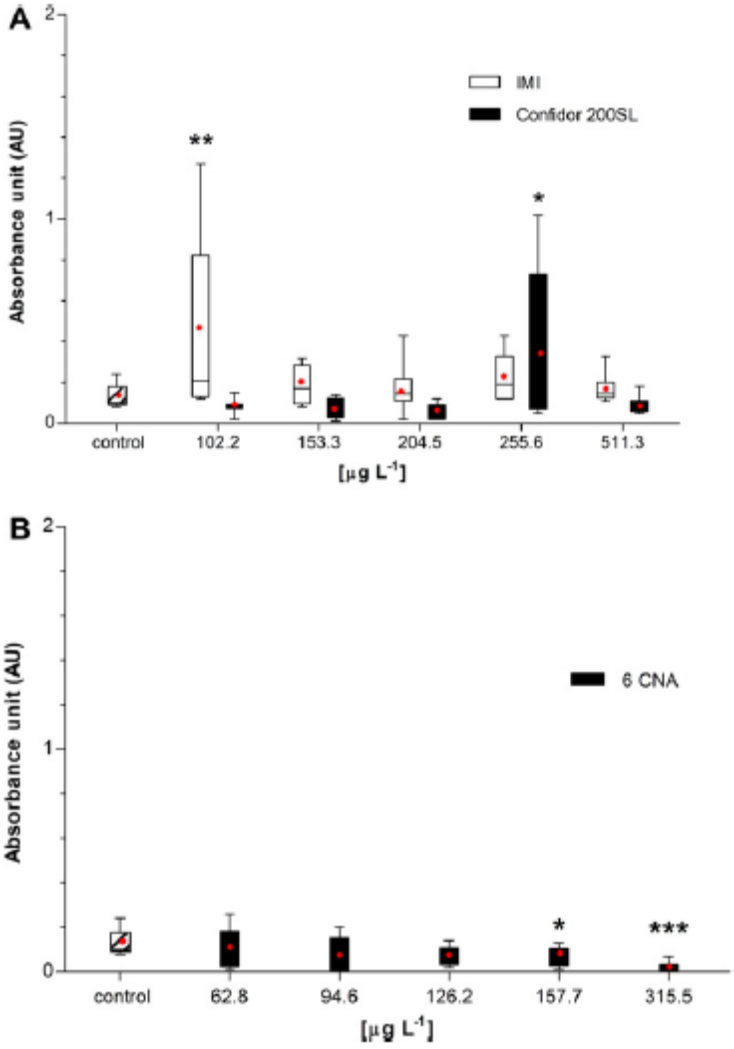


Figure 5: Whole-body Lipid Peroxidation of *G. fossarum* (expressed in absorbance units of TBARS products) measured after 24 hr of exposure IMI or Confidor 200SL (A) and 6 CNA (B). Boxes represent minimum and maximum values and (•) represents mean.



Overall assessment	Methodology, results and discussion were well documented. The study is considered a limited reliability because it was not performed

	in line with a recognised guideline and there is no information that it was performed in line with GLP.

Toxicity of new molecules of insecticides against honeybee *Apis mellifera* L.	
KCA 8.3.1	
Author(s)	Nadaf, H., Yadav, G.S., Kaushik, H.D., Sharma, K.
Year	2013
Journal	Trends in Biosciences Vol. 6(4), pp. 445-447
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch et al., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this study were to determine the effects of pesticides to the honeybee *Apis mellifera* L. Commercial grade formulations of Acetamiprid 20SP and other pesticides were used in the study. Mortality was assessed 2, 4, 6, 12 and 24 hr during exposure.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No specific methods were cited, however detailed methods are provided.
Test substance Identification of test substance, source, purity, stability	Commercial grade formulation of acetamiprid 20SP
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	27 ± 2 ºC
Controls Positive control, negative control	Negative control of water residue
Dosing system Exposure (dose, duration, frequency)	Rearing jars (10 x 7 cm diameter) were used as test chambers with one mL of test material applied to the inside of each jar. The formulation of test pesticides were diluted in 1 L of water and made into spray solutions. One mL of each pesticide was transferred to clean dry rearing jars. The jar was rotated and left overnight for drying. **Pesticide** | **Dose (g a.i./ha)** Acetamiprid 20SP | 10
Test species	Adult *Apis mellifera* L., honeybees, from the Research Apiary of

<table>
<tr><td>Body weight or length, gender, age/life stage, source</td><td>Department of Entomology, CCS Haryana Agricultural University.</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Ten bees per replicate with three replicates per concentration.

Completely Randomized Block Design was adopted.

Percent mortality was calculated and mortality observed in control treatment was adjusted using Abbott’s formula (Abbotts, 1925). Corrected mortality percentage was angular transformed and subjected for analysis of variance.</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>
<table>
<tr><th rowspan="2">Pesticide</th><th rowspan="2">Dosage (g a.i./ ha)</th><th colspan="5">Mean Percent Mortality After Treatment</th></tr>
<tr><th>2 h</th><th>4 h</th><th>6 h</th><th>12 h</th><th>24 h</th></tr>
<tr><td>Acetamiprid</td><td>10</td><td>0.00 (0.00)</td><td>0.00 (0.00)</td><td>21.93 (27.49)</td><td>84.90 (67.27)</td><td>100.00 (90.00)</td></tr>
</table>
Figures in parenthesis are angular transformation values.
</td></tr>
<tr><td>Overall assessment</td><td>The article included detailed methods, results and discussion, however it should be considered with ‘limited reliability’ due to the following concerns.
• A dilution series was not used in toxicity testing
• Concentrations used were not determined by chemical analysis.
• Study was not performed in line with a recognised guideline
• No indication that the study was performed in line with GLP</td></tr>
</table>

Zebrafish developmental screening of the ToxCast™ Phase I chemical library	
KCA 8.2	
Author(s)	Padilla, S., Corum, D., Padnos, B., Hunter, D.L., Beam, A., Houck, K.A., Sipes, N., Kleinstreuer, N., Knudsen, T., Dix, D.J. and Reif, D.M.
Year	2012
Journal	Reproductive Toxicology Vol. 33, pp. 174-187
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	Acetamiprid was tested in a zebra fish embryonic developmental assay. Embryos were exposed for five days. Lethality, hatching and malformation were assessed at the end of the exposure.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	• EPA zebra fish embryonic developmental assay for ToxCast
Test substance Identification of test substance, source, purity,	• Acetamiprid (a.s.)

stability	
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 26 ± 0.1°C • Photoperiod: 14:10 light: dark cycle • Water: freshwater
Controls Positive control, negative control	• Positive control: chlorpyrifos ethyl • Vehicle control: DMSO (0.4% v/v)
Dosing system Exposure (dose, duration, frequency)	• Test concentrations: Range from 0.001356 to 80 µM (24 hr water-renewal for 5 days) • Complete solution change with chemical renewal every 24 h
Test species Body weight or length, gender, age/life stage, source	• Zebrafish (*Danio rerio*) • Source: Aquatic Research Organisms, New Hampshire, USA • Age: embryos (6-8 hrs after fertilization)
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	• 4 embryo / test concentration for 5 days • Statistics: Standard sigmoidal curves were fit using a 4-parameter Hill model to determine the half-maximal activity concentrations (AC_{50}).
Biological effects Determined effect concentration, dose response observed	• Lethality, hatching and malformation were assessed at the end of the exposure. • Toxicity score: 0.5
Overall assessment	• Methodology, results and discussion are documented. • The statistical analysis used was described. • The study is considered reliable.

Toxicity of neonicotinoid insecticides to *Neoseiulus californicus* and *Phytoseiulus macropilis* (Acari: Phytoseiidae) and their impact on functional response to *Tetranychus urticae* (Acari: Tetranychidae).	
KCA 8.3.2	
Author(s)	Poletti, M., Maia, A.H.N., Omoto, C.
Year	2007
Journal	Biological Control Vol. 40, pp. 30-36
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this study were to evaluate the effect of neonicotinoid insecticides acetamiprid, imidacloprid and thiamethoxam on the survival of adult female *Neoseiulus californicus* and *Phytoseiulus macropilis*, non-target predatory mites, as well as

<table>
<tr><td></td><td>the impact on the functional response to Tetranychus urticae, the two-spotted spider mite, eggs. A residual-type bioassay was used to evaluate mortality of adult females and the functional response of predators when introduced to prey. All insecticides evaluated showed low toxicity on expose adult females. Acetamiprid did not affect the predatory capacity of N. californicus but it was detrimental to P. macropilis. Imidacloprid changed the functional response of both predator species and thiamethoxam significantly reduced P. macropilis consumption of prey.</td></tr>
<tr><td colspan="2">Reliability check: study details</td></tr>
<tr><td>Parameter</td><td>Information available</td></tr>
<tr><td>Test protocol
GLP, GEP, Guidelines (US EPA, OECD, …)</td><td>No specific protocol is cited but methods are well detailed.</td></tr>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td>
<table>
<tr><th>Compound</th><th>Commercial product</th><th>Concentration</th><th>Concentrations mg a.i./L</th></tr>
<tr><td>Acetamiprid</td><td>Mospilan</td><td>200 g a.i./kg</td><td>80</td></tr>
<tr><td>Imidacloprid</td><td>Confidor 700 WG</td><td>700 g a.i./kg</td><td>280</td></tr>
<tr><td>Thiamethoxam</td><td>Actara 250 WG</td><td>250 g a.i./kg</td><td>135</td></tr>
</table>
</td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>25 ± 2ºC, 70 ± 10% relative humidity and 14 hr photophase</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative control was distilled water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Adult female mortality
Three cm C. ensiformis leaf discs were sprayed with pesticides to deposit approximately 1.60 mg/cm^2. The leaf discs were left to dry and then placed on 3% agar-water mixture in a 3.5 cm diameter petri dish. Five females of each predatory mite species (Neoseiulus californicus and Phytoseiulus macropilis) were added to each leaf disc arena. Mortality was evaluated after 48 hr.

Impact on functional response
Leaf disc arenas, containing four C. ensiformis leafs discs of 3 cm diameter arranged on pieces of foam imbibed in water inside 12 cm petri dishes, were prepared with different densities of Tetranychus urticae eggs (5, 10, 20, 40, 60 and 80 eggs per arena). Insecticides were sprayed onto the leaf discs containing eggs to the same</td></tr>
</table>

	concentration in the mortality experiments. One predatory mite was added to each leaf disc arena. The effect on mean consumption of each predatory mite was evaluated after 24 hrs by counting the number of eggs consumed at each density.
Test species Body weight or length, gender, age/life stage, source	*Neoseiulus californicus* and *Phytoseiulus macropilis* were maintained on jack bean plants in a greenhouse at 25 ± 2ºC, 70 ± 10% relative humidity and 14 hr photophase. Toxicity tests were conducted with female adults under the same laboratory conditions.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	**Adult female mortality** Ten replicates for each treatment were used. Percent mortality was analysed by analysis of variance (ANOVA) and treatment means were compared to controls by Dunnett's test. **Impact on functional response** The experiment was replicated five times with five arenas per treatment. Insecticide effect on consumption of prey by predator was evaluated via ANOVA of eggs consumed per leaf disc arena. The effect of pesticide was analysed by comparing predator's mean consumption of eggs on pesticide treated leaf discs to consumption of eggs on leaf treated with water using the F-test. The mean variation rate in egg consumption by unit density increase (ΔNa) was calculated for each treatment (insecticides and control) using the following equation: $\Delta N\text{a} = ((Na_{N\max} - Na_{N\min}))/N_{\max} - \text{N}_{\min})$ Na = number of prey (*T. urticae* eggs) consumed by predator N_{min} = minimum density evaluated in the experiment N_{max} = maximum density evaluated in the experiment ΔNa = the mean slope of the curve which describes the variation in egg consumption as a function of the increase in prey density. Functional response model was used to describe the variation in the number of prey consumed by the predatory (Na) as a function of prey density (N) was calculated using equation: $Na = (a \bullet T \bullet N)/(1 + a\ (\text{Th} + c \bullet N.)N)$ a = attack coefficient c = function shape ($c = 0$ means that handling time is density-independent, while $c > 0$ implies that handling time increases with N) T = time, in this study 1 day Th = time used by predator for the identification, capture, consumption of prey. N = prey density. For this study $c = 0$ and a and Th were estimated using non-linear functional response models corresponding to each treatment (Gauss-

<table>
<tr><td></td><td>Newton iterative method.</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>All three test materials showed low toxicity on adult females of N. californicus and P. macropilis, based on the residual-contact bioassays. The mean percentage mortality for both species in the treatments involving insecticides did not differ significantly from the control (water) (p > 0.5)

Table 1: Toxicity to adult females
<table>
<tr><th rowspan="2">Treatments</th><th colspan="2">Percentage Mortality (± SE)</th></tr>
<tr><th>N. californicus</th><th>P. macropilis</th></tr>
<tr><td>Control</td><td>8.0 ± 4.2</td><td>8.0 ± 4.7</td></tr>
<tr><td>Acetamiprid</td><td>10.0 ± 3.0[a]</td><td>2.0 ± 3.3[a]</td></tr>
<tr><td>Imidacloprid</td><td>2.2 ± 3.1[a]</td><td>12.0 ± 3.3[a]</td></tr>
<tr><td>Thiamethoxam</td><td>6.0 ± 3.0[a]</td><td>6.0 ±3.3a</td></tr>
</table>
[a] Mean mortality did not differ from the control (Dunnett’s test, 0 > 0.5)

The number of prey consumed per predator increased quickly as the prey density offered initially increased, becoming levelled later with additional increases. Based on the functional response parameters evaluated, it was verified that the performance of N. californicus was only affected by imidacloprid, which reduced the mite’s attack coefficient (a) and increased prey handling time (Th). On the other hand, all neonicotinoids tested affected the functional response exhibited by P. macropilis. The peak consumption estimated for N. californicus was reduced by one half when the eggs were sprayed with imidacloprid, as compared with consumption in the control. Even though P. macropilis consumed up to 60 eggs/arena when they were sprayed with water (control), all neonicotinoids tested reduced the peak egg consumption by this predator. Reductions of approximately four, eight and two times were observed for acetamiprid, imidacloprid and thiamethoxam, respectively. Imidacloprid caused a reduction in N. californicus consumption, which was significant only when food availability was higher or equal to 40 eggs/arena. At these densities, N. californicus consumption was about six to eight times higher in the control than in the imidacloprid treatment. Acetamiprid did not decrease N. californicus consumption on treated eggs. Thiamethoxam affected this predator’s consumption only at the density of 40 eggs/arena. P. macropilis consumption on T. urticae eggs significantly decreased in the acetamiprid, imidacloprid and thiamethoxam treatments starting at densities of 20, 10 and 40 eggs per arena, respectively. A relation between mean variation in consumption (ΔNa) and handling time (Th) could be established. Both for N. californicus and P. macropilis, it was observed that the higher the ΔNa a, the lower the estimated Th. Greater variations in this parameter were observed for P. macropilis when the control was compared with neonicotinoid-sprayed eggs. This was due to the mite’s predatory capacity on T. urticae eggs, associated with the fact that consumption decreased as a consequence</td></tr>
</table>

	of all insecticides under study. **Table 2: Estimates of functional response parameters** **Species** / **Treatment** / **a^a (95% CL)** / **Th^b (95% CL)** *N. californicus* / Control / 0.86 (0.41-1.31) / 0.06 (0.04-0.07) *N. californicus* / Acetamiprid / 0.88 (0.14-1.62) / 0.08 (0.05-0.10) *N. californicus* / Imidacloprid / 0.51 (0.00-1.60) / 0.13[c] (0.07-0.19) *N. californicus* / Thiamethoxam / 0.81 (0.09-1.53) / 0.08 (0.05-0.11) *P. macropilis* / Control / 0.87 (0.48-1.25) / 0.02 (0.01-0.03) *P. macropilis* / Acetamiprid / 0.48 (0.15-0.81) / 0.07[c] (0.04-0.09) *P. macropilis* / Imidacloprid / 0.16 (0.03-0.29) / 0.14[c] (0.04-0.23) *P. macropilis* / Thiamethoxam / 0.63 (0.28-0.97) / 0.03 (0.01-0.04) [a] Attach coefficient [b] Handling time [c] Significantly different from controls at 5% level when 95% confidence intervals did not overlap
Overall assessment	The article included detailed methods, results and discussion, however it should be considered with 'limited reliability' due to the following concerns: • A dilution series was not used in the experiments. The pesticides were only tested at manufacturers' recommended application rates for the control of certain pests. • Chemical analysis of the pesticides was not conducted to determine actual concentrations. • Study was not performed in line with a recognised guideline • No indication that the study was performed in line with GLP

Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiiae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae) and *Neoseiulus cucumeris* (Acari: Phytoseidae).	
KCA 8.3.2	
Author(s)	Stara, J., Ourednickova, J., Kocourek, F.
Year	2011
Journal	Journal of Pest Science Vol. 84, pp. 25-31
Relevance check	Relevant
Reliability check	Reliable: 1 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	The goal of this study was to characterize the side effects of the six insecticides, including acetamiprid, to the parasitic wasp *Aphidius colemani*, the predatory gall midge *Aphidoletes aphidimyza* and the predatory mite *Neoseiulus cucumeris*. Acetamiprid caused 100% mortality in *A. colemani* 24 h after application, 48.9% mortality in *N. cucumeris* and 2.5% mortality in *A. aphidimyza*. In general, *N. cucumeris* exhibited the lowest sensitivity to all insecticides. In contrast, *A. colemani* was the most sensitive.

<table>
<tr><th colspan="2">Reliability check: study details</th></tr>
<tr><th>Parameter</th><th>Information available</th></tr>
<tr><td>Test protocol
GLP, GEP, Guidelines (US EPA, OECD, …)</td><td>The procedure that the International Organization for Biological Control of Noxious Animal and Plants IOBC recommends for the evaluation of side effects of plant protection products on non-target arthropods was used to test the side effects of insecticides to A. colemani. Methods for other toxicity tests are well detailed in the article.</td></tr>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td><table><tr><th>Compound</th><th>Active Ingredient</th></tr><tr><td>Mospilan 20SP</td><td>Acetamiprid 200 g/kg</td></tr></table></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>Temperature: 25ºC
Relative humidity: 65%
Photoperiod: 16:8 h light:dark</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: Non-treatment
Positive Control: Dimethoate</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Toxicity to A. colemani
Test units consisted of 10 x 10 cm test units consisting of two glass plates and a stainless steel frame with holes. The holes were covered with a fine mesh gauze. The glass plates were treated with 0.2 mL of insecticide solution using an adjustable spray wash bottle providing an application rate of 200 L/ha. After 10 min drying, organisms were introduced into the test chamber with ten organisms per test chamber, with four replicates per treatment and exposed for 48 h.

Toxicity to A. aphidimyza
Filter paper was treated with insecticide and then placed into Petri dishes. A. aphidimyza larvae (10 per replicate, 4 replicates per treatment) were introduced into the petri dishes and exposed to fresh residues for 24 hr and mortality then evaluated.

Toxicity to N. cucumeris
Filter paper was treated with insecticides and placed into 12-well tissue culture chambers. Ten mites per replicate, 4 replicates per treatment, were exposed for 24 hr and mortality then evaluated.

<table><tr><th>Compound</th><th>Active Ingredient</th><th>A.i. concentration (g/L H_2O)</th></tr><tr><td>Mospilan 20SP</td><td>Acetamiprid 200 g/kg</td><td>0.026</td></tr></table></td></tr>
</table>

<table>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Aphidius colemani, Aphidoletes aphidimyza and Neoseiulus cucumeris were obtained from Koppert B.V., Berkel en Rodenrijs, the Netherlands. A. colemani arrived as aphid mummies and incubated until hatch at 22ºC, 65% relative humidity and 16:8 hr light:dark photoperiod. Adult wasps were used within 48 hr after hatching and fed 1:3 v/v solution of honey water until test initiation and during the duration of the test. A. aphidimyza arrived as adults and placed onto potted bean plants and allowed to lay eggs. They fed on Acyrthosiphon pisum that were on the bean plants. They were kept in similar conditions as A. colemani and second instar larvae were used for experiments. N. cucumeris arrived as adults and used within 48 hr of delivery.</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>XL-STAT program was used for analysis of variance (ANOVA) on all mortality and A. colemani fecundity. Mortality rates were transformed using angular transformation (arcsin), the fecundity of each A. colemani was log transformed. Tukey’s post hoc comparison was used to find differences among the pesticides on 5% probability level in both mortality and fecundity studies.</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Mortality
Acetamiprid caused 100% mortality in A. colemani 24 h after application, 48.9% mortality in N. cucumeris and 2.5% mortality in A. aphidimyza.

Table 1: Percent mortality per species and corresponding category according to IOBC classification of pesticides
<table>
<tr><th>Insecticide</th><th>Species</th><th>Mortality 24 hr</th><th>IOBC Category</th></tr>
<tr><td rowspan="3">Acetamiprid</td><td>Nc</td><td>2.5</td><td>1</td></tr>
<tr><td>Aa</td><td>48.9</td><td>2</td></tr>
<tr><td>Ac</td><td>100</td><td>4</td></tr>
</table>
Nc = N. cucumeris; Aa = A. aphidimyza; Ac = A. colemani</td></tr>
<tr><td>Overall assessment</td><td>The article can be considered ‘limited reliability’ due to detailed methods, results and discussion with the exception of the following concerns:
• A dilution series was not used testing the pesticides. They were tested at their recommended field application rates.
• There is no analytical data to support the concentrations tested.</td></tr>
</table>

Plant protection product news, proof of toxicity in greenhouse bumblebees	
KCA 8.3.2	
Author(s)	Sterk, G., Benuzzi, M.
Year	2004
Journal	Protected Cultivation Vol. 1, pp. 75-77
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable

<table>
<tr><td>Summary</td><td>The study was performed to investigate the effects of insecticides on bumblebees via (i) contact toxicity tests, larvae feeding tests and adult feeding toxicity tests. Microbiology-based insecticides were completely harmless to bumblebees even when placed directly in contact with each individual. Acetamiprid was much less toxic that other neonicotinoid insecticides.</td></tr>
<tr><td colspan="2">Reliability check: study details</td></tr>
<tr><td>Parameter</td><td>Information available</td></tr>
<tr><td>Test protocol
GLP, GEP, Guidelines (US EPA, OECD, …)</td><td>No specific methods were cited, however methods followed Sterk et al (1995) and Merckx (2002).</td></tr>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td>Table 1: Test materials
<table>
<tr><th>Active Ingredient</th><th>Commercial Name</th><th>Formulation</th></tr>
<tr><td>Acetamiprid</td><td>Mospilan</td><td>20 SP</td></tr>
</table></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>No test conditions are cited</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Contact Toxicity Tests
50 µl of the commercial product solution (at recommended application) were given to individual worker bees through a micropipette.

Larvae Feeding Toxicity Tests
Methods followed those by Sterk et al (1995) and Merckx (2002). Treated pollen was fed ad libitum

Adult Feeding Toxicity
Methods followed those by Sterk et al (1995) and Merckx (2002). Treated sugar solution was fed ad libitum

Table 2: Doses
<table>
<tr><th>Active Ingredient</th><th>Commercial Name</th><th>Dosage of commercial formulation (%)</th></tr>
<tr><td>Acetamiprid</td><td>Mospilan</td><td>0.04</td></tr>
</table></td></tr>
</table>

Test species Body weight or length, gender, age/life stage, source	*Bombus terrestris L.* workers were used for contact and adult feeding toxicity tests, larvae were used in larvae feeding toxicity tests. No other information is provided.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	For direct contact toxicity tests, mortality is expressed as a percentage of workers surviving in comparison with the control. In both oral toxicity tests mortality was calculated according to the reduction in brood. Commercial products were classified according to categories proposed by International Organization for Biological Control (IOBC) working groups: Class 1, harmless (<25%), Class 2, slightly toxic (25-50%), Class 3, moderately toxic (51-75%) and Class 4, toxic (> 75%).
Biological effects Determined effect concentration, dose response observed	Microbiology-based insecticides were completely harmless to bumblebees even when placed directly in contact with each individual. Acetamiprid was much less toxic that other neonicotinoid insecticides. **Table 3: Toxicity values** **Substance** | **Toxicity Tests (IOBC Category)** | **Persistence** **Direct Contact** | **Larvae Feeding** | **Adult Feeding** Acetamiprid | 2 | 2 | 4 | 3 days Class 1: harmless (< 25% mortality); Class 2: slightly toxic (25-50% mortality); Class 3: moderately toxic (51-75% mortality); Class 4: toxic (> 75% mortality)
Overall assessment	Due to key deficiencies, this article should be considered with 'limited reliability': • There is no analytical data to support the concentrations tested. • Not complete reporting of crucial methods and data analysis • Study was not performed in line with a standardised guideline and there is no indication of GLP

Effect of insecticides on the mortalities of three whitefly parasitoid species, *Eretmocerus mundus, Eretmocerus eremicus and Encarsia formosa* (Hymenoptera: Aphelinidae)	
KCA 8.3.2	
Author(s)	Sugiyama, K., Katayama, H., Saito, T.
Year	2011
Journal	Applied Entomology and Zoology Vol. 46, pp. 311-317
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this research were to determine the toxicity of 24 insecticides to three parasitoid species; *Eretmocerus mundus, Eretmocerus eremicus* and *Encarsia formosa*. Neonicotinoids were seriously harmful to adult parasitoids. For each insecticide, the mortality of pupae was generally lower than that of adults.

<table>
<tr><th colspan="2">Reliability check: study details</th></tr>
<tr><th>Parameter</th><th>Information available</th></tr>
<tr><td>Test protocol
GLP, GEP, Guidelines (US EPA, OECD, …)</td><td>No specific protocol is cited, but detailed methodology is reported.</td></tr>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td>Table 1: Test materials
<table>
<tr><th colspan="2">Compound</th><th rowspan="2">Active Ingredient (%)</th></tr>
<tr><th>Chemical</th><th>Trade Name</th></tr>
<tr><td>Acetamiprid</td><td>Mospilan</td><td>20</td></tr>
</table></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>• Temperature: 25ºC
• Photoperiod: 16:8 h light:dark.
• Filter paper soaked in 5% honey solution was offered as food.</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>• Dry film toxicity test to Pupae
• Negative control: DI water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Dry film toxicity test to adults
0.1 mL of insecticide diluted in acetone was poured into test chambers and rotated to the coat the inner surface. Mortality was recorded after 24 hrs.

Dry film toxicity test to pupae
Insecticides were diluted in DI water to concentrations recommended by the manufacturer for use. Mummy cards containing parasitoid pupae were dipped in test solutions for 10 s. Three cards for each parasitoid species were used for each insecticide and control. After dipping, the mummy cards were dried on a paper towel and monitored for two weeks. Numbers of dead parasitoids were counted under a microscope.

Table 2: Dilutions
<table>
<tr><th colspan="2">Compound</th><th rowspan="2">Dilution</th></tr>
<tr><th>Chemical</th><th>Trade Name</th></tr>
<tr><td>Acetamiprid</td><td>Mospilan</td><td>x2,000</td></tr>
</table></td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>E. mundus, E. eremicus and E. formosa were purchased from Koppert B.V. and arrived in 80-100 parasitized whitefly pupae.

Adult toxicity tests: Mummy cards were incubated until the</td></tr>
</table>

	emergence of adults. 2-3 day old adults were used in toxicity tests. Pupae toxicity tests: Mummy cards containing 80-100 whitefly pupae parasitized with test species were used in toxicity tests.
Statistical analyses Sample size/replicates, statistical analysis of data (significance level, variability)	Ten adults were placed into a replicate, six replicates were used per treatment per species. Mortality was calculated with Abbott's formula and classified into 4 levels; 1) harmless, < 30% mortality; 2) slightly harmful, 30-79% mortality; 3) moderately harmful, 80-90% mortality; 4) Seriously harmful, > 99% mortality.
Biological effects Determined effect concentration, dose response observed	**Adult mortality** Mortalities from five different neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid and nitenpyram) were 100% for all three parasitoid species. **Pupae mortality** Treatment with acetamiprid resulted in a level 3 classification towards *E. eremicus* and a level 4 classification towards *E. mundus* and *E. formosa*.
Overall assessment	Due to key deficiencies, this article should be considered with 'limited reliability': • There is no analytical data to support the concentrations tested. • The specific concentration range for each insecticide is not adequately reported • No complete reporting controls • Study was not performed in line with a standardised guideline and there is no indication of GLP

Toxicity of Spirotetramat 150 OD to honeybees	
KCA 8.3.1	
Author(s)	Vinothkumar , B., Kumaran, N., Boomathi, N, Saravanan, P.A., Kuttalam, S.
Year	2010
Journal	The Madras Agricultural Journal Vol. 97, pp. 86-87
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al.*, 2007)
Reasons for no reliability	Not applicable
Summary	Laboratory studies were conducted to evaluate the toxicity of Spirotetramat 150 OD to honeybees using contact toxicity tests. Spirotetramat 150 OD tested at three different application rates caused less toxicity in Indian bees, Italian bees, little bees and stingless bees than acetamiprid.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No specific protocol is cited and method details are limited.

<table>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td>Acetamiprid 20 SP</td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>No details are provided</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: distilled water</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Toxicity tests were carried out in plastic containers perforated to provide aeration. Treated filter paper (1 mL insecticide solution) was placed inside the container and allowed to dry for 15 min. Ten bees were placed in each test chamber and after 1 hr the insecticide treated filter paper was removed and a 40% sucrose solution soaked cotton wool was provided as food. Mortality was assessed at 6, 12 and 24 hr.

<table>
<tr><th>Insecticide</th><th>Dose (g a.i./ha)</th></tr>
<tr><td>Acetamiprid 20 SP</td><td>20</td></tr>
<tr><td>Untreated Control</td><td></td></tr>
</table>
</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>• Apis cerana indica Fabb. - Indian bee
• Apis mellifera Linn - Italian bee
• Apis florea F. - Little bee
• Trigona iridipennis - Stingless bee

All bees were worker bees, no age reported.</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>• Nine treatments with three replicates for each treatments were used in a completely randomise design.
• Percent mortality was calculated</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Acetamiprid mortality
<table>
<tr><th rowspan="2">Species</th><th colspan="3">Mortality*</th></tr>
<tr><th>6 hr</th><th>12 hr</th><th>24 hr</th></tr>
<tr><td>Sting less</td><td>21.14^{b}</td><td>33.00^{b}</td><td>37.22^{b}</td></tr>
<tr><td>Little bee</td><td>26.07^{bc}</td><td>33.00^{bcd}</td><td>41.5^{d}</td></tr>
<tr><td>Indian</td><td>28.78^{c}</td><td>31.00^{de}</td><td>41.15^{cd}</td></tr>
<tr><td>Italian</td><td>26.07^{cd}</td><td>31.00^{cd}</td><td>43.08^{de}</td></tr>
</table>
*Data has been arc sine transformed. Means followed by a common letter are not significantly different (p = 0.05)</td></tr>
</table>

Overall assessment	The article can be considered 'limited reliability' due to detailed methods, results and discussion with the exception of the following concerns: • A dilution series was not used testing all the pesticides. They were tested at their recommended field application rates. • There is no analytical data to support the concentrations tested. • The article was not well detailed in the methods and data analysis of the research. • Study was not performed in line with a standardised guideline and there is no indication of GLP

Comparative acute toxicity of twenty-four insecticides to earthworm, *Eisenia fetida*	
KCA 8.4	
Author(s)	Wang, Y., Cang, T., Zhao, X., Yu, R., Chen, L., Wu, C., Wang, Q.
Year	2012b
Journal	Ecotoxicology and Environmental Safety Vol. 79, pp. 122-128
Relevance check	Relevant
Reliability check	Reliable: 1 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this research were to determine the toxic effects of twenty-four insecticides on earthworms and to provide informative data for use in ecological risk assessment on soil ecosystems. Results of the contact filter paper toxicity bioassay indicate that neonicotinoids were supertoxic to *E. fetida*, pyrethroids were very toxic and insect growth regulators were moderately toxic. Antibiotics, carbamates and organophosphates induced variable toxicity and were very to extremely toxic. Soil toxicity bioassays showed a different pattern of toxicity except that neonicotinoids were still the most toxic class of chemicals tested. The acute toxicity of neonicotinoids was higher than antibiotics, carbamates, growth inhibitors and organophosphates.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Modified Organization for Economic Co-operation and Development (OECD 1983) and International Standardization Organization (ISO) earthworm toxicity test.
Test substance Identification of test substance, source, purity, stability	Acetamiprid, 97% technical grade (a.i.)
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of	**Contact Filter Paper Test** 20 ± 1 ºC, kept in the dark. **Artificial Soil Test** 10% ground sphagnum peat (< 0.5 mm), 20% kaolinite clay (> 50% kaolinite) and 70% fine sand (OECD, 1984). pH was adjusted to 6.0 ± 0.5 with calcium carbonate. Water content was adjusted to 35% of the

<table>
<tr><td>animals, food availability</td><td>dry weight. Test conditions were 20 ± 1ºC, 80-85% relative humidity, 400-800 lx of constant light.</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Contact Filter Paper Test Negative Control
Acetone

Artificial Soil Test Negative Control
Acetone</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Contact Filter Paper Test
A piece of filter paper was treated with the test substance dissolved in 2 mL of acetone in a 9 cm petri dish. After the solvent evaporated, the piece of filter paper was remoistened with 2 mL distilled water and one organism was placed on it. The exposure lasted 48 hr and then mortality was recorded.

Artificial Soil Test
Insecticide was dissolved in 10 mL acetone and mixed into a small quantity of fine quartz sand. The sand was mixed for 1 hr to evaporate the acetone and then mixed with the artificial soil (10% ground sphagnum peat, 20% kalinite clay, 70% fine sand). 0.65 kg soil was placed into 500 mL glass jars. Mortality was assessed at 7 and 14 days after treatment.</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Earthworms, Eisenia fetida, purchases from College of Animal Sciences, Zhejiang University, China weighing between 350 and 500 mg</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Contact Filter Paper Test
One worm per replicate, 10 replicates per treatment, five treatments plus control.

Artificial Soil Test
Ten adult worms per replicate, three replicates per treatment, six treatments plus control.

Statistical analysis
A probit analysis was used to assess acute toxicity. Significant differences were based on non-overlap between the 95% confidence limits of two LC50 values ($p < 0.05$). Contact filter paper acute toxicity LC50 values were classified as supertoxic (< 1.0 µg/cm^2), extremely toxic (1-10 µg/cm^2), very toxic (10-100 µg/cm^2), moderately toxic (100-1000 µg/cm^2), or relatively nontoxic (> 1000 µg/cm^2).</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Acute toxicity from contact filter paper test
<table>
<tr><th>Insecticide</th><th>LC50 (95% CL) µg/cm^2</th><th>Toxicity Grade</th></tr>
<tr><td>Acetamiprid</td><td>0.0088 (0.0066-0.011)</td><td>Supertoxic</td></tr>
</table>
Toxicity Grade: Supertoxic (< 1.0 µg/cm^2), extremely toxic (1-10 µg/cm^2), very toxic (10-100 µg/cm^2), moderately toxic (100-1000 µg/cm^2) or</td></tr>
</table>

	relatively nontoxic (> 1000 µg/cm^2). Acetamiprid was determined to be the most toxic substance tested. **Table 1: Acute toxicity from artificial soil test** **Insecticide** | **7 day LC50 (95%CL) mg/kg** | **14 day LC50 (95%CL) mg/kg** Acetamiprid | 1.72 (1.58-1.97) | 1.52 (1.41-1.67) Acetamiprid was determined to be the most toxic substance tested at both 7 and 14 days.
Overall assessment	Reliable study investigating earthworm toxicity performed in line with standardised guidelines.

Insecticide toxic effects on *Trichogramma ostriniae* (Hymenoptera: Trichogrammatidae)	
KCA 8.3.2	
Author(s)	Wang, Y., Chen, L., Yu, R., Zhao, X., Wu, C., Cang, T., Wang, Q.
Year	2012c
Journal	Pest Management Science Vol. 68, pp. 1564-1571
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of the study were to examine the toxic effects of selected insecticides on *Trichogramma ostriniae* (parasitic wasp) under laboratory conditions. Among the seven classes of insecticides organophosphates and carbamates had the highest toxicity. They are followed by phenylpyrazoles, avermectins, neonicotinoids and pyrethroids. The growth inhibitors exhibited the least toxicity. Risk quotient analysis classifies neonicotinoids, avermectins, pyrethroids, growth inhibitors and phenylpyrazoles as safe against the test species.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	Methods by Desneux et al. (2006) were cited
Test substance Identification of test substance, source, purity, stability	**Insecticide** | **Technical Grade (A.I%)** | **Recommended Field Rate (g a.i./ha)** Acetamiprid | 97 | 18-22.5
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light	• Temperature: 25 ± 1 ºC • 70 ± 10% relative humidity • 14:10 h light:dark photoperiod. Organisms were given access to honey as food.

<table>
<tr><td>intensity, number of animals, food availability</td><td></td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: acetone</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Acetone solutions of insecticides were made and 500 µl of solution was allowed to cover the internal surface of glass tubes used as test chambers (8.0 cm h, 2.0 cm diameter, 53.38 cm^2 surface area). Tubes were rotated to cover the glass wall and then left for 1 hr at room temperature to dry. After 1 h exposure the wasps were transferred into clean tubes with access to honey as food. After 24 hr dead organisms were counted.</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>Trichogramma ostriniae adults 24-48 hr after emergence. Organisms were from an in-house culture kept according to methods detailed in Preetha et al. (2010).</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>80-100 wasps were placed in each replicate, three replicates were used for each dose.

Percent mortality was corrected using the Abbott’s formula. This data was then analysed by probit analysis suing EPA Probit Analysis Program v. 1.5. A significant level of two LC50 values was subjected to probit analysis using the POLO program.

Risk quotients were calculated from LC50 values 24 hr after treatment, based on the following formula: risk quotient = field-recommended rate (g a.i./ha) / LC50 (mg a.i./L).</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Median lethal concentrations
<table>
<tr><th>Insecticide</th><th>LC50 (95%FI; mg a.i./L)</th><th>LC95 (95%FI; mg a.i./L</th><th>Risk Quotient</th><th>Category</th></tr>
<tr><td>Acetamiprid</td><td>43.02 (34.99-55.70)</td><td>383.0 (233.9-782.6)</td><td>0.52</td><td>1</td></tr>
</table>
Category: 1) Safe; 2) Slightly to Moderately Toxic; 3) Dangerous</td></tr>
<tr><td>Overall assessment</td><td>The article can be considered reliable due to detailed methods, results and discussion. However, due to the following deficiencies, this article should be considered with ‘limited reliability’:
• There is no analytical data to support the concentrations tested.
• The specific concentration range for each insecticide is not reported.
• Study was not performed in line with a standardised guideline and there is no indication of GLP</td></tr>
</table>

Susceptibility to selected insecticides and risk assessment in the insect egg parasitoid *Trichogramma confusum* (Hymenoptera: Trichogrammatidae)
KCA 8.3.2

Author(s)	Wang, Y., Chen, L., An, X., Jiang, J., Wang, Q., Cai, L., Zhao, X.
Year	2013b
Journal	Journal of Economic Entomology Vol. 106(1), pp. 142-149
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this research were to determine the toxicity of several different insecticides to *Trichogramma confusum*. Among the seven classes of tested chemical, organophosphates and carbamates exhibit the highest toxicity to the test species. This is followed by phenylpyrazoles, avermectins, pyrethroids and neonicotinoids. In contrast, insect growth regulators showed the least toxicity. A risk quotient analysis indicated that neonicotinoids (except thiamethoxam), avermectins, pyrethroids, growth inhibitors and phenylpyrazoles are safe, but organophosphates and carbamates are slightly, moderately or dangerously toxic to *T. confusum*.
Reliability check: study details	
Parameter	**Information available**
Test protocol GLP, GEP, Guidelines (US EPA, OECD, …)	No specific protocol is cited, however detailed methods are provided.
Test substance Identification of test substance, source, purity, stability	**Insecticide** / **Recommended field concentration (mg a.i./L)** Acetamiprid / 27-33.75
Test conditions Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability	• Temperature: 25 ± 1ºC • 70 ± 10% relative humidity • 14:10 h light:dark photoperiod. Wasps were given access to honey as food.
Controls Positive control, negative control	Negative control: acetone
Dosing system Exposure (dose, duration, frequency)	Acute contact toxicity tests were conducted by coating glass vials (height x diameter, 8.0 cm x 2.0 cm) with 500 µl of test solution. The tubes were rotated until the glass walls were coated and left to dry at room temperature for 1 hr. Adult wasps (80-100) were added and after 1 hr of exposure the wasps were then moved into clean insecticide-free test chambers with honey for food. After 24 hr, mortality was counted. 675 L/ha was used as a comparison between recommended field concentrations of insecticides and acute toxicity.
Test species Body weight or length,	Adult *Trichogramma confusum* 24-48 hr old were used in the study and were from an in-house culture.

<table>
<tr><td>gender, age/life stage, source</td><td></td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>80-100 organisms per test chamber.

Percent mortality was corrected by using the Abbott formula. The data were then analysed via probit analysis using EPA Probit Analysis Program v. 1.5 and the log concentration probit mortality line (Finney, 1971). Significant differences between means were based on the nonoverlap between 95% confidence limits (CL) of LC50 values.

Risk quotients were calculated from LC50 values 24 hr after treatment, based on the following formula: risk quotient = field-recommended rate (g a.i./ha) / LC50 (mg a.i./L).</td></tr>
<tr><td>Biological effects
Determined effect concentration, dose response observed</td><td>Table 1: Median lethal concentrations
<table>
<tr><th>Insecticide</th><th>LC50 (95%FI; mg a.i./L)</th><th>Risk Quotient</th><th>LC95 (95%FI; mg a.i./L</th></tr>
<tr><td>Acetamiprid</td><td>93.21 (83.26-106.1)</td><td>0.24 (*)</td><td>675.4 (515.7-938.2)</td></tr>
</table>
Category: * = safe, ** = slightly to moderately toxic, *** = dangerously toxic

Out of 30 insecticides tested, acetamiprid was twenty-third in order of toxicity.</td></tr>
<tr><td>Overall assessment</td><td>The article can be considered reliable due to detailed methods, results and discussion. However, due to the following deficiencies, this article should be considered with ‘limited reliability’:
• There is no analytical data to support the concentrations tested.
• The specific concentration range for each insecticide is not reported.
• Study was not performed in line with a standardised guideline and there is no indication of GLP</td></tr>
</table>

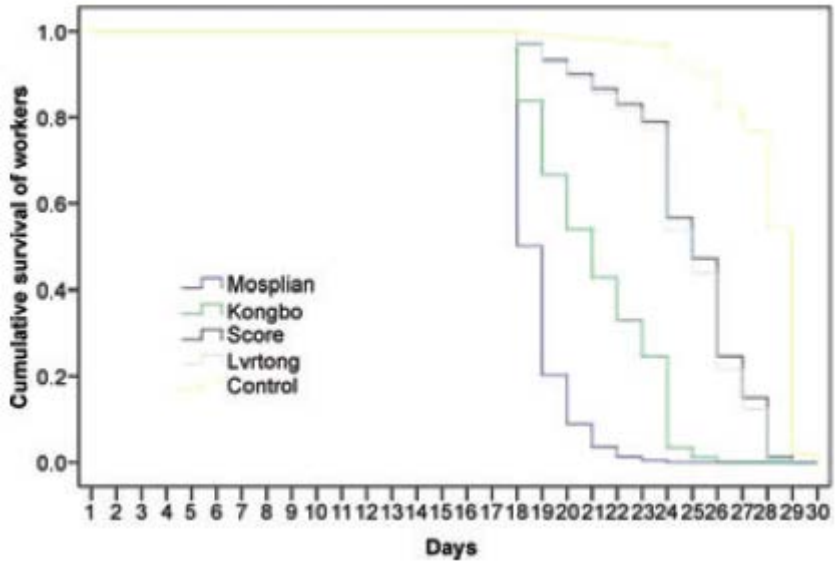
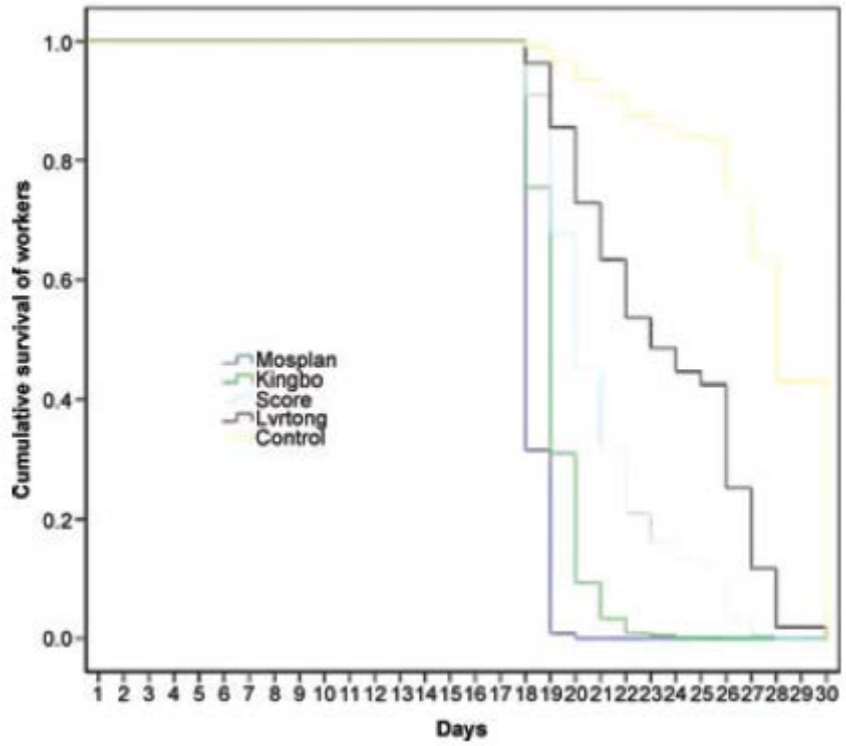
Sensitivities of three bumblebee species to four pesticides applied commonly in greenhouses in China	
KCA 8.3.1	
Author(s)	Wu, J., Li, J-L., Peng, W-J., Hu, F-L.
Year	2010
Journal	Insect Science Vol.17, pp. 67-72
Relevance check	Relevant
Reliability check	Reliable: 2 (Klimisch *et al*., 2007)
Reasons for no reliability	Not applicable
Summary	The objectives of this study were to determine the toxicity of widely used pesticides to several bee species via contact and ingestion tests. The results showed that mortality of *Bombus hypocrita* after contacting the four pesticides was significantly lower than *Bombus patagiatus* and *Bombus ignitus*. The oral toxicity median lethal dose

<table>
<tr><td></td><td>(LD50) value of Mospilan to B. hypocrita was significantly higher than that to B. ignitus and B. patagiatus. Of the bee species it can be concluded that B. hypocrita was the most robust of the test species. The mortality rates of each species were significantly higher after contact with Mospilan than the control group.</td></tr>
<tr><td colspan="2">Reliability check: study details</td></tr>
<tr><td>Parameter</td><td>Information available</td></tr>
<tr><td>Test protocol
GLP, GEP, Guidelines (US EPA, OECD, …)</td><td>No specific protocol is cited, but detailed methods are provided.</td></tr>
<tr><td>Test substance
Identification of test substance, source, purity, stability</td><td><table><tr><th>Compound</th><th>Active Ingredient (%)</th></tr><tr><td>Mospilan</td><td>Acetamiprid (3)</td></tr></table></td></tr>
<tr><td>Test conditions
Temperature, pH, oxygen concentration, water hardness, conductivity, photoperiod, light intensity, number of animals, food availability</td><td>Temperature: 27ºC
60% relative humidity.

Organisms were fed 50% w:w sugar syrup and water during the course of contact toxicity tests experiment via gravity feeders replaced every two days.</td></tr>
<tr><td>Controls
Positive control, negative control</td><td>Negative Control: 50% sugar solution without pesticide for ingestion tests.</td></tr>
<tr><td>Dosing system
Exposure (dose, duration, frequency)</td><td>Contact Test
The bottom of a test chamber (40 x 40 x 40 cm) was covered with paper sprinkled with 20 mL of test solution and air-dried. Organisms were monitored up to 16 days post initial contact.

Ingestion Test
Initial studies determined a proper dilution series of five concentrations, plus control, that were prepared in 50% sugar solutions. Prior to offering the test solution, organisms were starved for 2-3 hr. Each test solution of 10 mL was provided in vertical feeders. Mortality was monitored after 48 hr.</td></tr>
<tr><td>Test species
Body weight or length, gender, age/life stage, source</td><td>B. hypocrita, B. ignitus and B. patagiatus queens were captured and kept in wooden nest boxes in a climate room (28-29ºC and 60-65% relative humidity) and fed with 50% sucrose solution and pollen. Workers, aged 9-10 days after emergence, were selected at random from different colonies for the contact test.</td></tr>
<tr><td>Statistical analyses
Sample size/replicates, statistical analysis of data (significance level, variability)</td><td>Thirty workers per replicate were tested in three replicates per treatment in both tests.

Statistical analysis was done with SPSS 17.0 software. Treatments were compared among species, treatment groups and the control group. A Cox-survival analysis was done. Mortality rates were</td></tr>
</table>

	analysed using ANOVA with Tukey's post-hoc test. The LD50 was calculated with POLO-PC software.
Biological effects Determined effect concentration, dose response observed	**Figure 1: Survival of *B. patagiatus* up to 30 days post contact with pesticides**  **Figure 2: Survival of *B. ignites* up to 30 days post contact with pesticides**  **Figure 3: Survival of *B. hypocrita* up to 30 days post contact with pesticides**

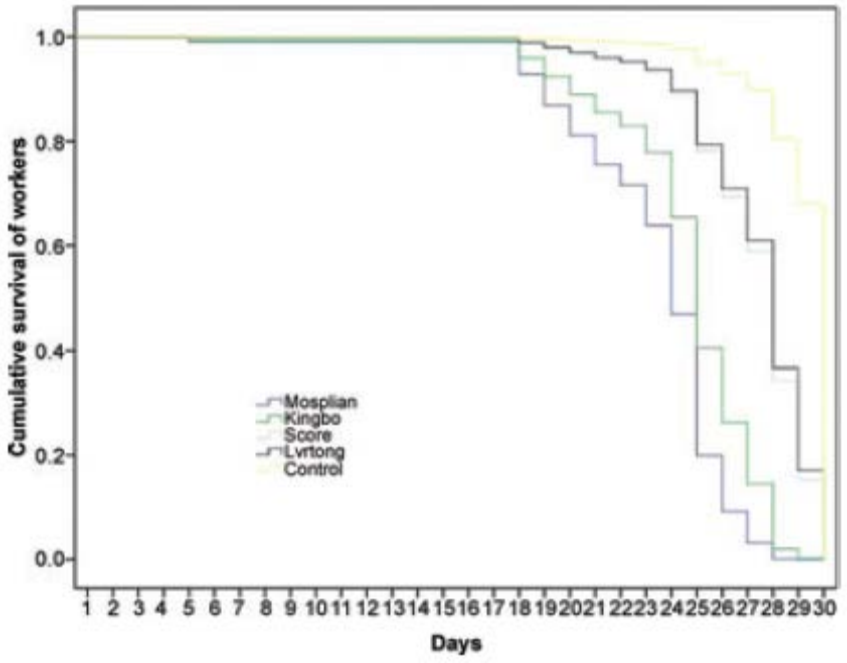


Figure 4: Percentage of mortality of the three bee species after 16 days of contact with each pesticide

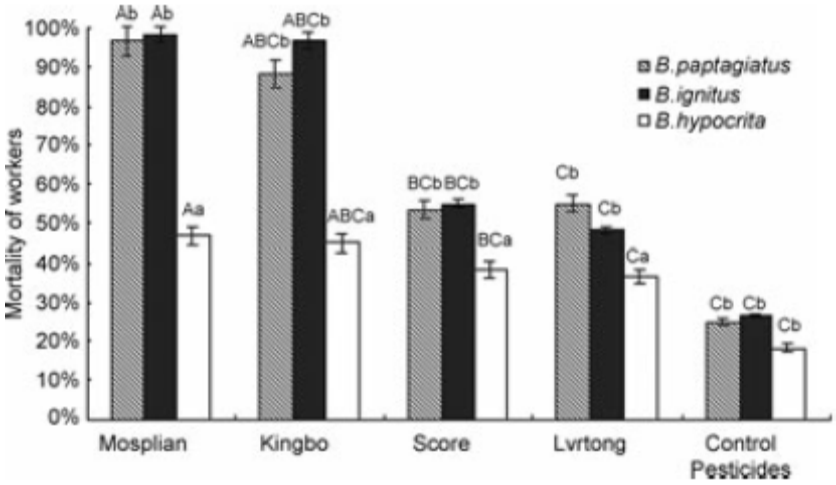


Table 1: Oral toxicity of Mospilan to bumblebee species

Species	LD50 (95% CL) (µg A.I./bee)
Bombus ignites	0.0023 (0.0021-0.0024)
Bombus hypocrite	0.0028 (0.0018-0.0031)
Bombus patagiatus	0.0021 (0.0020-0.0023)

Overall assessment

The article can be considered reliable due to detailed methods, results and discussion. However, due to the following deficiencies, this article should be considered with 'limited reliability':

- There is no analytical data to support the concentrations tested.
- The specific concentration range for each insecticide is not reported.

	• Not complete reporting of some methods and data analysis • Study was not performed in line with a standardised guideline and there is no indication of GLP.